THE
HÆMOLYTIC ANÆMIAS

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Congenital and Acquired

By

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PREFACE

My aim in writing a book on the hæmolytic anæmias has been to present in a volume of moderate size a comprehensive and up-to-date account which I hope will prove of value to both physicians and pathologists. The book is larger than was contemplated. Its size could have been reduced by describing only those blood disorders in which an increased rate of hæmolysis is known to be a major factor in pathogenesis and excluding conditions such as Mediterranean anæmia in which increased hæmolysis is of much less importance. However this course had to be abandoned because of the difficulty of drawing a hard and fast distinction between the two categories.

The hæmolytic anæmias have a large and world-wide literature and to review all that has been written on the subject would be a superhuman task. Most of the papers referred to in the present text have been published in the last ten to fifteen years. Nevertheless I have attempted to do justice to the pioneer workers of earlier generations by *referring to the papers in which their* more important discoveries were described.

The plan of the book has been to give in an introductory chapter a brief survey of the hæmatology of increased hæmolysis and the ways in which the hæmolytic anæmias may be investigated. Then follow five chapters on the congenital hæmolytic anæmias and six chapters on the acquired hæmolytic anæmias associated with auto-antibody formation. Subsequent chapters deal with the secondary hæmolytic anæmias, paroxysmal nocturnal hæmoglobinuria and hæmolytic disease of the newborn. In the last chapter are described the laboratory methods that I use in the study of hæmolytic disorders. The text includes, particularly in Chapter 9, unpublished material taken from a thesis submitted in 1952 to the University of London for the degree of M.D. (Pathology).

Although the book has been written from the standpoint of a pathologist and is thus mainly concerned with hæmatological and serological observations and with problems of pathogenesis the clinical side of the picture has not been neglected. Moreover the clinical and laboratory findings in thirty personally investigated patients suffering from different types of hæmolytic anæmia have been included, and the findings in five other patients

accounts of which have already been published given in more detail or brought up to date I have been fortunate in the wonderful collaboration that I have received from the Medical Staff of the Postgraduate Medical School of London in the study of these patients and I am grateful for permission to report clinical details of the patients under their care I am also indebted to Drs P Ellman and S D V Weller for allowing me to report the clinical histories of the patients referred to as Cases 5 and 7 respectively

It is a pleasure to record my appreciation of the encouragement that I have received from Professor J H Dible and the help which has been given by members of his staff Dr J C White and Dr J G Selwyn in particular have kindly allowed me to quote from work which has not yet been published Dr White has also contributed a section included in Chapter 18 on physico chemical methods useful in the investigation of abnormal hæmoglobins and Professor E J King has allowed me to quote extensively also in Chapter 18 from his book Microanalysis in Medical Biochemistry in describing methods for the estimation of bilirubin and urobilinogen I am also greatly indebted to Drs J H Crookston G A W Johnston D L Mollin L S Sacker J G Selwyn and J C White for reading the typescript or proofs of the book in whole or in part and for making many valuable suggestions and to Drs D I Mollin P L Mollison and Dorothy Parkin for permission to quote unpublished data on erythrocyte survival Dr Parkin and Miss Marie Cutbush have also helped me greatly by genotyping the blood of certain patients Finally I should like to record my sincere appreciation of the generosity of many other friends amongst physicians and pathologists who have given me permission to investigate patients under their care or who have sent me samples of blood

The photomicrographs were taken by Mr E V Willmott F R P S and Mr C A P Graham Figs 1 9 10 11 12 14 15 27 38 42 43 50 65 66 90 and 91 were taken by Mr Willmott the remainder by Mr Graham The black and white figures were drawn by Miss Patricia Simms I am much indebted to their patience and skill

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J V DACIE

CONTENTS

CHAPTER 1

GENERAL FEATURES OF INCREASED HÆMOLYSIS: BLOOD PICTURE AND METHODS OF INVESTIGATION OF THE HÆMOLYTIC ANÆMIAS

The essential feature of a hæmolytic anæmia is a reduction of the life span of the patient's erythrocytes. As will be shown later this may be due to many different causes. This chapter is concerned with the ways in which an increased rate of erythrocyte destruction *in vivo* may be recognized and with the methods which may be used to measure the intensity of increased hæmolysis. The processes which bring about destruction of erythrocytes in health will also be briefly considered. Finally, the significance and importance of certain laboratory tests will be discussed in connection with the diagnosis of hæmolytic disease.

DESTRUCTION OF ERYTHROCYTES IN HEALTH

It is now generally accepted that in health approximately 1/120th of the total number of circulating erythrocytes is destroyed and replaced daily. This figure is based upon estimates of the average life span of the normal erythrocyte which have been derived from several different types of experiment (Callender, Powell and Witts, 1945; 1947; Jope, 1946; Shemin and Rittenburg, 1947). However, the way in which erythrocytes are destroyed and eliminated from the circulation in health is largely an unsolved problem.

Various suggestions have been made as to how this is brought about. Erythrophagocytosis by phagocytes in the spleen and elsewhere, although undoubtedly an important mechanism in disease, does not seem to be an adequate explanation by itself for the normal physiological blood destruction (Rous, 1923). It is possible that towards the end of the normal life span of about 100 to 130 days the erythrocytes break up into small fragments which are subsequently removed from the circulation by reticulo-endothelial cells in the spleen and elsewhere. Evidence for the presence of fragmenting erythrocytes (schistocytes) was furnished by Rous and Robertson (1917). More recent evidence which

might be held to support this contention has been provided Stewart Stewart Izzo and Young (1950) who making use corpuscles labelled with ^{59}Fe showed that the mechanical fragili of the oldest cells increased before they were eliminated from t circulation

The changes in the erythrocyte or at its surface which cau fragmentation or increased sensitivity to mechanical trauma a quite obscure Possibly the changes are connected with a wearin out of enzyme systems which control metabolic activities essenti for the integrity of either the cell as a whole or of its membrar (Grannick 1949 Ponder 1951) The expulsion of sodium ior against a concentration gradient in the presence of glucose ma be quoted as one such essential activity (Flynn and Maizels 1949 It is also known that certain constituents of the erythrocyt stroma and membrane are in a state of dynamic exchange wit constituents in the plasma This appears to be true of bot cholesterol and phospholipids and London and Schwarz (195 suggested that death of the erythrocyte might follow from th loss of the metabolic lability of some normally labile constituen or be due to the loss of the stability of constituents such as h globin and stromal protein which are normally metabolic stable

It is possible too that influences outside the cell play a pa in bringing about the cumulative damage which limits the life the normal erythrocyte It has been suggested for instanc that stagnation of the blood stream particularly in the splee might be deleterious (Fahraeus 1939 Ham and Castle 194(and b) and it is conceivable that tissue lysins normally inhibite by plasma may play a part under conditions of stasis (Ponde 1951) Normal plasma too is known to contain potential aut agglutinins and lysins active at 37 C against erythrocyt damaged by enzymes such as trypsin (Rosenthal and Schwart 1951 Hurley and Dacie 1953) or against defective erythrocyt like those of paroxysmal nocturnal hæmoglobinuria It is co: ceivable that normal corpuscles although apparently insensitiv to these agglutinins and lysins in crude *in vitro* tests are signi: cantly affected *in vivo* where they are exposed to the action these factors for much longer periods of time

Catabolism of Hæmoglobin *There seem to be two ma* channels for the disposal of hæmoglobin liberated by erythrocyt destruction If a cell or a fragment of a cell is taken up by a erythrophage in the spleen or elsewhere in the body (extravascul lysis) the hæm of the hæmoglobin molecule becomes transforme

rubin which is eventually eliminated from the circulation liver and finally forms a major part of the urobilinogen of ces The iron and protein part of hæmoglobin are retained body This is probably the main method by which hæmo- n is disposed of in health If on the other hand as in certain lytic anæmias the erythrocyte breaks up or is lysed in the l stream (intravascular lysis) the liberated hæmoglobin is sed of in two ways part passes through the renal glomeruli if in sufficient concentration appears in the urine and part roken down in the plasma liberating hæmatin which when ed with plasma albumin forms the brown pigment methæm- umin (Fairley 1941) The pigment moiety of methæmalbumin robably excreted by the liver as bilirubin (Pass Schwartz and tson 1945 London 1950)

IDENCE FOR AN INCREASED RATE OF HÆMOLYSIS

As the bile pigments and fæcal urobilinogen are largely derived m the catabolism of hæmoglobin it is natural to expect ased production and elimination of these substances whenever rate of erythrocyte destruction is increased

Hyperbilirubinæmia In hæmolytic anæmia the plasma ilirubin concentration usually lies between 1 and 3 mg per 00 ml Occasionally it is within the normal range it is rarely bove 5 mg per 100 ml The direct van den Bergh reaction is usually negative in uncomplicated cases The bilirubin concen tration however is an unreliable measure of hæmolysis as it depends not only on the amount of pigment produced but also on the efficiency of the liver in excreting it Moreover the total amount produced depends not only on the rate of hæmolysis but also upon the total number of erythrocytes present For instance the same amount of bilirubin might be expected to be produced per day by the destruction of 5% of a patient s erythrocytes when the total count was 5 000 000 per c mm as by the destruction of 25% of the erythrocytes when the count was 1 000 000 per c mm Other things being equal therefore the highest bilirubin levels might be expected in patients with the highest erythrocyte counts In practice however this expected correlation is seldom found as the patients with the highest counts are usually those in whom the rate of hæmolysis is not great i e they are patients in whom compensation for hæmolysis is possible (see p 16)

It is probable that in those patients in whom the plasma bili rubin level is normal despite evidence of increased hæmolysis the

normal levels are maintained by the ability of the healthy liver to excrete far more bilirubin than it is normally called upon to do

Excretion of Urobilinogen The quantitative estimation of the fæcal excretion of 'urobilinogen'—the name given to the fæcal pigments reducible to chromogens reacting with Ehrlich's reagent (Gray 1953)—has been widely used as a measure of hæmolysis (see Watson 1938 Crosby and Akeroyd 1952) It is true that in hæmolytic anæmia the excretion of pigment is often far in excess of normal but the inaccuracies and uncertainties of the estimation are such that it can hardly be expected to provide reliable evidence of a slight increase in hæmolysis The technical difficulties of the collection of twenty four hour or four day excretions of fæces difficulties in obtaining representative samples of the specimens and the use of an arbitrary colour standard in the actual estimation all combine to reduce the reliability of the estimations There are also difficulties in interpretation over and above the purely technical difficulties Although it has been shown in dogs with artificial biliary fistulæ given acetyl phenylhydrazine that 88% on an average of the hæm liberated by the breakdown of hæmoglobin is recovered in the bile (Cruz Hawkins and Whipple 1942) it is not certain whether this is true in man Moreover it is known that the amount of pigment that can be estimated as fæcal urobilinogen is considerably lower than the bilirubin excretion This suggests that either the conversion of bilirubin into urobilinogen is not quantitative or else that the urobilinogen is altered in part into other substances which are not readily estimated (Watson 1942 Crosby and Akeroyd 1952 Gray 1953) Furthermore reabsorption of a proportion of the fæcal pigments certainly takes place The fate of the reabsorbed pigments is not known with certainty it is surmised that most of the pigment is re excreted in the bile unless there is liver disease when some appears in the urine as urobilinogen and urobilin

Another unexpected cause of discrepancies between amounts of hæmoglobin catabolized and fæcal urobilinogen excreted has recently been discovered Studies with the ^{15}N isotope have shown that a significant proportion of the fæcal hæm pigment is derived from sources other than hæmoglobin (London West Shemin and Rittenburg 1950 Gray Neuberger and Sneath 1950) In health this proportion may be as high as 10–20% in pernicious anæmia it may be as high as 40% (London and West 1950) It is thought that the non hæmoglobin derived pigment comes from at least two sources from hæm or porphyrins not

utilized to form hæmoglobin and from myoglobin (London West Shemin and Rittenburg 1950 Gray Neuberger and Sneath 1950)

In view of the difficulties and uncertainties mentioned above it is hardly surprising that fæcal urobilinogen estimations carried out on normal subjects give widely divergent figures For example the normal range of the daily excretion by an adult is from 40 to 280 mg per day according to Watson and Bilden (1941) and Watson (1942) and from 22 to 121 mg per day according to Maclagen (1946) while estimations carried out by Sparkman (1939) on single specimens of stool from 100 normal subjects gave results varying from 76 4 to 520 mg per 100 g of fæces Normal values for children are given by Mills and Mason (1952)

There is a further practical point of interpretation which has to be taken into account The total daily urobilinogen excretion of a child is normally much less than that of an adult because the total amount of circulating hæmoglobin is far less The urobilinogen excretion of an anæmic man will be less than that of a man without anæmia for the same reason The only satisfactory way to get round this difficulty is to relate the pigment excretion to the total circulating hæmoglobin expressing the result as so many milligrams of urobilinogen per 100 g hæmoglobin (see Watson 1938) In health this amounts to 11-21 mg per 100 g hæmoglobin per day (Miller Singer and Dameshek 1942) The theoretical ratio based on the known molecular weights of hæmoglobin (68 000) and four molecules of urobilinogen (2 000) and assuming that a 1/120th of the circulating hæmoglobin is broken down every day is 24 mg per 100 g hæmoglobin In practice the total amount of hæmoglobin in the body has to be computed from an estimate or guess of the patient s blood volume and this is often an additional source of error For all these reasons the estimation of fæcal urobilinogen may fail to give decisive information in the investigation of those mild examples of hæmolytic anæmia in which an accurate estimation of the rate of hæmoglobin breakdown is most needed

Urobilinogen in Urine A darkening of the urine particularly on standing due to excess of urobilin is frequently found in cases of hæmolytic anæmia The quantitative estimation of the pigment cannot however be used as a reliable index of erythrocyte destruction Urobilinuria is an indication that the liver is unable to re excrete urobilinogen reabsorbed from the bowel rather than a sign of increased hæmolysis (Watson 1937 Barker 1938)

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Hæmoglobinæmia and Hæmoglobinuria In normal

plasma there is very little free hæmoglobin probably less than 4 mg per 100 ml (Crosby and Dameshek 1951) In those types of hæmolytic anæmia in which hæmolysis takes place predominantly in the blood stream the plasma hæmoglobin may rise to 100–200 mg per 100 ml or even more In such cases hæmoglobinuria develops and the loss of pigment in this way may account for a substantial proportion of the total pigment excretion The mechanism of hæmoglobinuria is considered at length in Yuile s (1942) review It seems likely that hæmoglobin passes through the glomeruli even at low plasma concentrations only to be reabsorbed by the renal tubules If the plasma concentration exceeds 135 mg per 100 ml the concentration in the glomerular filtrate exceeds the capacity of the renal tubules for reabsorption and overt hæmoglobinuria normally results When hæmoglobin is absorbed by renal tubular cells the molecules of hæmoglobin are apparently broken down intracellularly as hæmosiderin granules giving Perls s reaction for free ferric iron appear In cases of chronic hæmoglobinæmia this leads to a striking degree of *siderosis of the kidneys (Fig 65 p 176) This loading of the* tubular cells with iron apparently impairs in time the cells capacity for absorbing more hæmoglobin for in chronic cases of hæmoglobinuria pigment may be present in the urine with plasma hæmoglobin levels well below the normal renal threshold of 135 mg per 100 ml (Gilligan Altschule and Katersky 1941)

The incidence and severity of hæmoglobinæmia in the hæmolytic anæmias have recently been reviewed by Crosby and Dameshek (1951) In addition to finding high values in the well known types of hæmoglobinuria they reported slightly raised concentrations in several types of acquired hæmolytic anæmia (without hæmoglobinuria) but not in congenital spherocytosis in overt sickle cell anæmia but not in sickle cell trait and in severe Mediterranean anæmia (thalassæmia major) but not in the trait (thalassæmia minor) It must be emphasized that the presence of excess hæmoglobin in the plasma is only a reliable sign of hæmolysis if the observer can be sure that the lysis has not been caused during or after the withdrawal of the blood

Methæmalbuminæmia Methæmalbumin was first observed by Fairley and Bromfield (1934) in the plasma of a patient suffering from blackwater fever The plasma was brownish in colour and spectroscopic examination showed an absorption band in the red part of the spectrum (at 624mμ) Subsequently Fairley (1941) showed that methæmalbumin could be detected in the plasma in several types of hæmolytic anæmia with hæmoglobinuria when

hæmolysis was taking place within the blood stream e g in blackwater fever in paroxysmal nocturnal hæmoglobinuria and in *Cl Welchii* septicæmia In addition he found the pigment to be present in smaller amounts in the plasma of patients with hæmolytic anæmia of unknown origin without hæmoglobinuria and in three patients suffering from typical severe pernicious anæmia On the other hand tests for the pigment were negative in two patients with hereditary spherocytosis and only very weakly positive in a third

Methæmalbumin is distinct chemically and spectroscopically from methæmoglobin (absorption band at 630mμ) It is a hæmatin albumin compound and is formed as a result of the degradation of hæmoglobin liberated into plasma Methæm albumin if not detectable by the position of its characteristic but rather faint absorption band in the red can be demonstrated by covering the serum or plasma with ether and then adding a one-tenth volume of concentrated ammonium sulphide This results in the formation of a hæmochromogen with a relatively intense sharply defined α absorption band at 558mμ (Schumm s test)

Fairley s work is now generally accepted and the presence of methæmalbumin demonstrated in serum by spectroscopy or by a positive Schumm s test is probably a reliable indication of intra vascular hæmolysis However the exact way in which the pig ment is formed *in vivo* from hæmoglobin remains obscure The pigment moiety of methæmalbumin is probably eventually transformed into bilirubin and excreted by the liver (Pass Schwartz and Watson 1945 London 1950)

Hæmosiderinuria The presence of brownish granules in the urine giving Perls s reaction for free ionic ferric iron is characteristic of the chronic hæmoglobinurias The iron is derived from hæmoglobin absorbed from the glomerular filtrate and subse quently broken down within the renal tubular cells As long ago as 1911 Marchiafava and Nazari recognized a granular form of hæmoglobin in the urine of a case of paroxysmal nocturnal hæmoglobinuria Rous (1918) observed hæmosiderin in the urine of a patient with acquired hæmolytic anæmia and in the urine of several patients suffering from pernicious anæmia who had received many transfusions Later Marchiafava (1928) referred to paroxysmal nocturnal hæmoglobinuria as Anemia emolitica con emosiderinuria perpetua Perpetual hæmosiderinuria is in fact a reliable sign of chronic intravascular hæmolysis for the urine will be found to contain iron granules even if there is no hæmo globinuria at the time Hæmosiderin is not however found in the

urine at the first onset of a hæmolytic attack even if accompanied by hæmoglobinuria as the pigment has to be absorbed by the tubular cells of the kidney and re excreted a process which occupies several days at least (Yule 1942)

The incidence and significance of hæmosiderinuria have been re investigated by Crosby and Dameshek (1951) They found hæmosiderin in the urine of every patient whose plasma continuously contained abnormal amounts of hæmoglobin and observed in general a parallelism between the amount of urinary hæmosiderin and the degree to which the plasma hæmoglobin level was raised Only very small amounts were found in the urine of patients whose plasma hæmoglobin levels were less than 20 mg per 100 ml on the other hand with plasma hæmoglobin levels greater than 40 mg the amount of hæmosiderin was as a rule sufficient to give a visible Prussian blue coloration to the urinary deposit when Perls s reaction was carried out

THE BLOOD PICTURE IN HÆMOLYTIC ANÆMIA

Evidence for Hæmolysis Important evidence of increased blood destruction may be obtained by examination of the blood itself Certain (prehæmolytic) abnormalities of the erythrocytes are probably almost always associated with an increased rate of hæmolysis The most important of these abnormalities are *spherocytosis* and *schistocytosis* (fragmentation)

Spherocytosis

Spherocytes are erythrocytes which are more nearly spheroidal and less distinctly disc like than are normal cells It should be emphasized that all grades of spherocytosis are met with ranging from cells which retain their biconcavities but whose thickness or breadth is increased to cells which are almost spherical As a rule the volume of a spherocyte is normal (see Table 1 p 17) hence any increase in thickness or breadth of the cell must be associated with a diminution in diameter Spherocytes are thus usually correctly called microspherocytes In stained blood films they appear as small usually perfectly round cells staining relatively intensely with Romanowsky dyes and usually showing no central pallor (Fig 1) Their unusual rotundity can also be recognized in wet preparations of fresh blood The increased density of staining is due not only to their shape but also to a slightly increased concentration of hæmoglobin (Table 1 and see p 64)

Spherocytes have to be differentiated from *spherical forms* According to Ponder (1948) spherical forms were first observed by Hamburger who in 1895 noted that when mammalian erythrocytes were suspended in saline or sugar containing media they appeared not as discs but as spheres This change can be reversed by the addition of plasma or serum to the suspension Furchgott (1940) showed that the sphering change was facilitated by the increase in pH which occurs when a thin layer of a cell suspension is placed between two glass surfaces as in ordinary microscopy and by the adsorption to glass of an anti sphering substance normally present in plasma Furchgott and Ponder (1940) showed that this substance was an albumin

This disc sphere transformation has by now been thoroughly investigated The stages in the process are disc crenated disc crenated sphere finely-crenated sphere and sphere (Ponder 1948) This sequence of events is also brought about by a wide range of hæmolytic agents including saponin brilliant green and amboceptor-complement in sublytic concentrations (Ponder 1948) In lytic concentrations the lysins cause the cells to become prolytic spheres and finally to fade from view as hæmolysis proceeds The sequence of changes as it occurs in saline suspensions of erythrocytes kept between glass surfaces is reversible As the cell becomes more and more nearly spherical its surface membrane becomes puckered—hence the crenated appearance—and eventually when the cell is spherical presumably thickened When the process is reversed the thickening and crenations disappear and the normal condition of the cell surface is restored

Spherocytes differ from *spherical forms* in several important ways the change to spheroidicity is *not* preceded by crenation the process is *not* reversible and complete sphering is rarely seen In addition spherical forms are not typically fragile to hypotonic saline (Ponder 1937 Gillespie 1943) spherocytes are

The association between spherocytosis and increased fragility to hypotonic saline (osmotic fragility) is well known (Haden 1934 Castle and Daland 1937 Guest 1948 Crosby 1952) The more nearly spheroidal a cell the less water it can absorb from hypotonic media without stretching its inextensible surface membrane Hence spherocytes will undergo lysis in media of a lesser degree of hypotonicity than do normal more discoidal cells of the same volume Castle and Daland (1937) and Guest (1948) have shown that spherocytes from cases of hereditary spherocytosis and normal corpuscles swell to a similar degree when placed

elliptocytosis in one family In seven of Holst Larsen's patients there was evidence of anæmia the elliptocytic erythrocytes were admixed with small rounded and irregularly shaped micro spherocytes in the more anæmic patients Another example of this type of abnormality was recorded by Dacie Mollison Richardson Selwyn and Shapiro (1953 Case 11) In this patient numerous very small irregularly shaped microspherocytes were conspicuous in films after splenectomy (Fig 40 p 98)

The 'Acquired' Spherocyte Spherocytes morphologically identical with those of hereditary spherocytosis i e rounded microspherocytes are frequently conspicuous in the blood of patients suffering from acquired hæmolytic anæmias associated with auto antibody formation (Fig 1 p 12) Spherocytes of apparently similar type may be readily produced experimentally in animals by the administration of heterospecific hæmolytic sera (see Chapter 9) Erythrocytes which have adsorbed anti A may also become spherocytic This change has been observed following the transfusion to group A patients of group O plasma containing the 'immune' type of anti A (Ervin and Young 1950 Ervin Christian and Young 1950) and in hæmolytic anæmia of the newborn when the mother is group O and the child group A (Grumbach and Gasser 1948 Crawford Cutbush and Mollison 1953) In each case the spherocytosis appears to be due to damage to the cell surfaces causing irreversible shrinkage

The osmotic and mechanical fragilities of acquired spherocytes are increased whether or not they swell to the same extent as hereditary spherocytes in hypotonic media is uncertain On incubation at 37 C *in vitro* acquired spherocytes undergo a variable and sometimes increased rate of autohæmolysis (Dacie 1950 Selwyn and Dacie 1954 see also p 174)

Spherocytes of apparently similar character have been observed in hæmolytic anæmias due to drug sensitivity e g sulphonamide hæmolytic anæmia (Gilligan and Kapnick 1941 Ross and Paegel 1946 see Chapter 15) A mild to moderate degree of spherocytosis is not uncommon in chronic myeloid leukæmia and myelosclerosis (see p 334) The significance of the change is not yet understood

Irregularly Contracted Erythrocytes Shrunken and distorted erythrocytes are not uncommonly seen in the blood stream in certain types of hæmolytic anæmia Following the ingestion of hæmolytic poisons such as acetylphenylhydrazine contracted corpuscles of irregular outline are conspicuous (Fig 2) Similarly distorted corpuscles have been observed by Brookfield

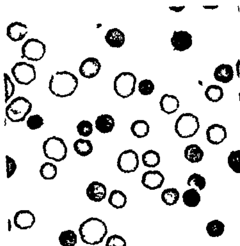


FIG 1 Photomicrograph of a blood film of a patient suffering from idiopathic acquired hæmolytic anæmia (Case 11) The contrast between the darkly staining spherocytes and the polychromatic larger reticulocytes is well shown × 00

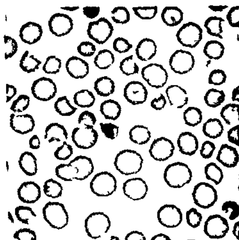


FIG 2 Photomicrograph of a blood film of a patient suffering from polycythæmia who had been treated with acetylphenylhydrazine The irregularly contracted darkly staining corpuscles contrast markedly with the less affected more lightly staining cells × 00

elliptocytosis in one family In seven of Holst Larsen's patients there was evidence of anæmia the elliptocytic erythrocytes were admixed with small rounded and irregularly shaped micro spherocytes in the more anæmic patients Another example of this type of abnormality was recorded by Dacie Mollison Richardson Selwyn and Shapiro (1953 Case 11) In this patient numerous very small irregularly shaped microspherocytes were conspicuous in films after splenectomy (Fig 40 p 98)

The 'Acquired' Spherocyte Spherocytes morphologically identical with those of hereditary spherocytosis i e rounded microspherocytes are frequently conspicuous in the blood of patients suffering from acquired hæmolytic anæmias associated with auto antibody formation (Fig 1 p 12) Spherocytes of apparently similar type may be readily produced experimentally in animals by the administration of heterospecific hæmolytic sera (see Chapter 9) Erythrocytes which have adsorbed anti A may also become spherocytic This change has been observed following the transfusion to group A patients of group O plasma containing the immune type of anti A (Ervin and Young 1950 Ervin Christian and Young 1950) and in hæmolytic anæmia of the newborn when the mother is group O and the child group A (Grumbach and Gasser 1948 Crawford Cutbush and Mollison 1953) In each case the spherocytosis appears to be due to damage to the cell surfaces causing irreversible shrinkage

The osmotic and mechanical fragilities of acquired spherocytes are increased whether or not they swell to the same extent as hereditary spherocytes in hypotonic media is uncertain On incubation at 37° C *in vitro* acquired spherocytes undergo a variable and sometimes increased rate of autohæmolysis (Dacie 1950 Selwyn and Dacie 1954 see also p 174)

Spherocytes of apparently similar character have been observed in hæmolytic anæmias due to drug sensitivity e g sulphonamide hæmolytic anæmia (Gilligan and Kapnick 1941 Ross and Paegel 1946 see Chapter 15) A mild to moderate degree of spherocytosis is not uncommon in chronic myeloid leukæmia and myelosclerosis (see p 334) The significance of the change is not yet understood

Irregularly-Contracted Erythrocytes Shrunken and distorted erythrocytes are not uncommonly seen in the blood stream in certain types of hæmolytic anæmia Following the ingestion of hæmolytic poisons such as acetylphenylhydrazine contracted corpuscles of irregular outline are conspicuous (Fig 2) Similarly distorted corpuscles have been observed by Brookfield

(13.8) in acute lead poisoning by Stats, Wasserman and Rosenfield (1948) in hæmolytic anæmia due to sulphapyridine and by Zuelzer and Apt (1949) in anæmia due to naphthalene (moth ball) poisoning. The cell distortion presumably results from the direct action of the poison upon the erythrocyte. An increase in osmotic fragility indicates that the cell membrane *itself* is damaged. This type of erythrocyte distortion although typical of that produced by some hæmolytic poisons may rarely be observed in hæmolytic anæmias of endogenous origin, e.g. in the hæmolytic anæmias associated with methæmoglobin and/or sulphæmoglobin formation (Fig. 3, see also p. 401).

Another somewhat different type of contracted corpuscle is not infrequently seen in anæmia complicating severe toxic states such as uræmia or carcinomatosis (Fig. 4). The abnormal cells appear to be undergoing irregular crenation and contraction and some of the cells are often almost triangular in outline (Fig. 5). The largest numbers are found in patients whose anæmia appears to be at least *in part* hæmolytic in origin.

These curiously deformed cells appear likely, therefore, to be prehæmolytic forms; reticulocytes are not affected. The osmotic fragility of blood containing triangular cells is usually normal or normal except for a very small tail of fragile cells (see p. 370). The cause of this type of change is obscure; presumably an abnormal metabolite is responsible.

The corpuscles referred to in the preceding paragraph are possibly the same as the burr cells which Schwartz and Motto (1949) observed in small numbers in various blood disorders and in larger numbers in uræmia, carcinoma of the stomach and bleeding peptic ulcer.

Schistocytosis

The products of erythrocyte fragmentation were referred to by Ehrlich (1891) as schistocytes and by Rous and Robertson (1917) as schizocytes. Such cell fragments are seldom seen in preparations of normal human blood. When visible in appreciable numbers their presence is good evidence of a hæmolytic process (Fig. 6). Normally it seems likely that any fragments formed are sieved out of the circulation by the spleen (see Robertson and Rous, 1917). Heating of blood *in vitro* and severe burns *in vivo* are known to cause erythrocyte fragmentation (Shen, Ham and Fleming, 1943; Brown, 1946; Ham, Shen, Fleming and Castle, 1948). In human patients the fragments resulting from severe burns disappear from the circulation within a few hours.



Fig. 3. Photomicrograph of a blood film of a patient suffering from a *hæmolytic anæmia of obscure origin* associated with methæmoglobinæmia and sulphæmoglobinæmia. Many irregularly contracted corpuscles can be seen. × 700.

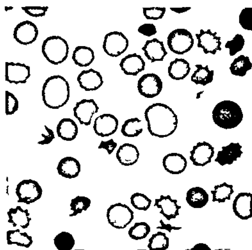


Fig. 4. Photomicrograph of a blood film of a patient suffering from carcinomatosis and hæmolytic anæmia (Case 2). Many irregularly crenated corpuscles are present (? "burr" cells, see p. 13). × 700.

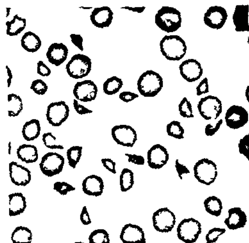


Fig [illegible] Photomicrograph of a blood film of a patient suffering from a (?) congenital hæmolytic anæmia with thrombocytopenia and uræmia (Case 1[illegible] of Dacie *et al* 19[illegible]3) Many triangular corpuscles are present × [illegible]00

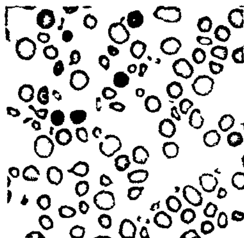


Fig [illegible] Photomicrograph of a blood film of a child suffering from a congenital hæmolytic anæmia (*Case 11 of Dacie et al 19[illegible]3*) Numerous microschistocytes can be seen as well as spherocytes × [illegible]00

Occasionally in cases of hæmolytic anæmia a few of the erythrocytes *appear as if part of their substance had been indented and* pulled outwards by means of a pair of pincers (Fig 7) Dacie and co workers (1953) observed these "pincered" cells in quite large *numbers in a patient suffering from slightly atypical hereditary* spherocytosis they were found in smaller numbers in other types of hæmolytic anæmia Presumably they too represent *corpuscles in the process of fragmentation* Rous and Robertson (1917) observed similar cells in normal rabbit blood and larger numbers in blood from rabbit spleen

Erythrophagocytosis

Human erythrocytes which have undergone phagocytosis by monocytes or neutrophils are rarely found in blood films Nevertheless they have been seen from time to time in small numbers in many types of hæmolytic disorder such as that associated with chemical poisoning septicæmia and protozoal infections and hæmolytic disease of the newborn paroxysmal cold hæmoglobinuria and idiopathic acquired hæmolytic anæmia (Jordan Prouty Heinle and Dingle 1952 Zinkham and Diamond 1952) (Fig 8) Zinkham and Diamond showed that the number of phagocytes containing erythrocytes might be greatly increased if the patient's blood was incubated *in vitro* at 37 C for 30 to 120 minutes before films were made It seems possible that in man at least phagocytes containing erythrocytes are rapidly removed from the circulation perhaps particularly by the spleen and lungs In animals such as the rat however erythrophago*cytosis in the peripheral blood is a marked feature of experi*mentally induced hæmolytic anæmia (Bessis and Freixa 1947) Bonnin and Schwartz (1954) have made a detailed study of the ability of different types of antibodies to cause erythrophagocytosis *in vitro* It was found that only those antibodies which were capable of causing hæmolysis in the presence of complement regularly caused erythrophagocytosis Monocytes appeared to be more active as erythrophages than neutrophils for the latter only enveloped corpuscles which had been sensitized by high concentrations of antibody

Heinz Bodies

Riess in 1882 noticed unusual rounded globules and granules in the erythrocytes in potassium chlorate poisoning Similar intracorpuscular bodies were later described in greater detail by Heinz (1890) in the blood of guinea pigs poisoned with pyrodin

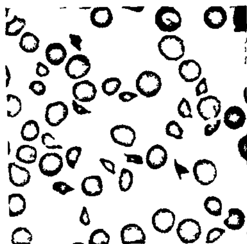


Fig 5 Photomicrograph of a blood film of a patient suffering from a (?) congenital hæmolytic anæmia with thrombocytopenia and uræmia (Case 1 of Dacie *et al* 1953) Many triangular corpuscles are present × 00

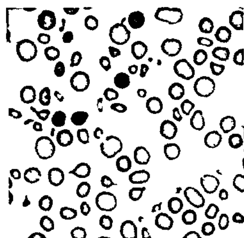


Fig 6 Photomicrograph of a blood film of a child suffering from a congenital hæmolytic anæmia (Case 11 of Dacie *et al* 1953) Numerous microschistocytes can be seen as well as spherocytes × 700

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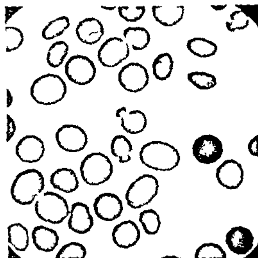


Fig 7 Photomicrograph of a blood film of a man suffering from a hæmolytic anæmia of unknown type associated with hæmoglobinuria Several pincered cells undergoing fragmentation can be seen × 1 000



Fig 8 Photomicrograph of a blood film from a patient suffering from idiopathic acquired hæmolytic anæmia showing erythrophagocytosis (Case 12) × 1 000

(acetylphenylhydrazine). Heinz bodies' are now known to be produced by the action on the blood of a wide range of aromatic nitro- and amino-compounds, as well as by inorganic oxidizing agents such as potassium chlorate. Heinz bodies may be found in the absence of anæmia, but large doses of all the drugs that cause Heinz body formation will cause anæmia. Recent reviews on Heinz bodies include those of Webster (1949) and Ruckell and Richardson (1950).

Heinz bodies probably consist of denatured globin derived from hæmoglobin itself. In the case of acetylphenylhydrazine it is thought that hæmoglobin acts as a catalyst in the oxidation of the acetylphenylhydrazine and is itself broken down in the process. Methæmoglobin is not usually formed under conditions which favour Heinz body formation (Beaven and White, 1954).

Morphologically, Heinz bodies are refractile rounded bodies, often with a slightly irregular contour, ranging in size from minute particles to bodies up to 3μ in size (Fig. 9). Several small bodies may be present in the same cell; the largest ones are usually present singly. They are easily visible in unstained or wet preparations of blood; they stain supravitally with a range of basic dyes including methyl violet, used by Heinz himself, and brilliant cresyl blue. However, they are not usually discernible in Romanowsky stained preparations or in brilliant cresyl blue-stained films fixed in methanol before counter-staining. They are generally only seen in fully ripened corpuscles and not in reticulocytes (but see p. 401). In cresyl blue-stained preparations they stain a distinctly lighter shade of blue than the reticular filamentous material of reticulocytes.

Heinz bodies occur in the blood in larger numbers after splenectomy (Zadek and Burg, 1930; Webster, 1949). It is possible that corpuscles containing Heinz bodies are selectively retained by the spleen or even that the spleen removes Heinz bodies from intact erythrocytes. Heinz body anæmia in man is considered in Chapter 15 (p. 399).

COMPENSATORY ERYTHROPOIESIS IN HÆMOLYTIC ANÆMIA

In hæmolytic anæmia the output of new erythrocytes from the marrow usually increases in step with the increased rate of hæmolysis. In this way some measure of compensation for the hæmolysis is generally achieved. In most instances when the hæmolytic process is a chronic one, a fairly steady balance between

destruction and formation is established at an erythrocyte level below the normal Occasionally compensation is complete and the patient manages to maintain a normal erythrocyte count and hæmoglobin level In severe hæmolytic states adequate compensation may be impossible with the result that the patients rapidly become seriously anæmic

The increase in erythropoiesis is brought about by *hyperplasia of the bone marrow* *The erythroid/myeloid ratio in the marrow* rises from the normal average proportion of about 1 in 5 to 1 in 1 in extreme instances erythropoietic cells may actually predominate (Fig 10) The fat cells in the marrow tend to disappear so that the marrow may become solidly cellular (Fig 11) The volume of active marrow increases and red marrow develops in the long bones and in sites in adults where it does not normally occur Occasionally, centres of extramedullary formation are found (see p 70)

In children the possibilities of hyperplasia of the marrow are more limited as nearly all the medullary cavities are normally occupied by active hæmopoietic marrow If the stimulus for increased erythropoiesis is sufficiently great hyperplasia may then result in an actual increase in the size of the medullary cavities In the skull this may lead to an obvious widening of the diploe Extramedullary foci are probably more frequent in children for the same reason

The hyperplasia of erythropoietic marrow is reflected in certain definite changes in the peripheral blood picture These changes are *reticulocytosis macrocytosis* and *erythroblastæmia*

Reticulocytosis in Hæmolytic Anæmia The normal range for the reticulocyte count is usually given as between 0 2% and 2 0% in adults In hæmolytic anæmia the proportion is often as high as 20% occasionally the count is much higher and may reach 70% or even more (Fig 12) Although there is no very close correlation between reticulocyte counts and the degree of anæmia the highest counts are generally found in the more anæmic patients in whom hæmolysis is usually more intense and efforts at compensation consequently greater

Failure of Regeneration Occasionally the erythropoietic activity of the bone marrow may fail in the course of a chronic hæmolytic anæmia with the result that the reticulocyte count falls to very low levels A serious increase in anæmia may result Dramatic examples have been described by Owren (1948) Dameshek and Bloom (1948) and Gasser (1950) in hereditary spherocytosis (see Chapter 2) and by Singer Motulsky and Wile



Fig 9 Photomicrograph of a blood film of a patient suffering from acetylphenylhydrazine poisoning (after splenectomy) (Case 31) Nearly every corpuscle contains a large Heinz body Stained supravitally by methyl violet × [illegible]00

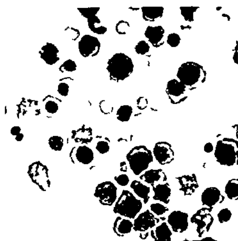


Fig 10 Photomicrograph of a film of sternal bone marrow from a patient suffering from paroxysmal nocturnal hæmoglobinuria (Case 3[illegible]) Normoblasts in all stages of development are the predominating cells × 400

[To face p 16

destruction and formation is established at an erythrocyte level below the normal. Occasionally compensation is complete and the patient manages to maintain a normal erythrocyte count and hæmoglobin level. In severe hæmolytic states adequate compensation may be impossible, with the result that the patients rapidly become seriously anæmic.

The increase in erythropoiesis is brought about by hyperplasia of the bone marrow. The erythroid/myeloid ratio in the marrow rises from the normal average proportion of about 1 in 5 to 1 in 1; in extreme instances erythropoietic cells may actually predominate (Fig. 10). The fat cells in the marrow tend to disappear so that the marrow may become solidly cellular (Fig. 11). The volume of active marrow increases, and red marrow develops in the long bones and in sites in adults where it does not normally occur. Occasionally centres of extramedullary formation are found (see p. 70).

In children the possibilities of hyperplasia of the marrow are more limited, as nearly all the medullary cavities are normally occupied by active hæmopoietic marrow. If the stimulus for increased erythropoiesis is sufficiently great, hyperplasia may then result in an actual increase in the size of the medullary cavities. In the skull this may lead to an obvious widening of the diploe. Extramedullary foci are probably more frequent in children for the same reason.

The hyperplasia of erythropoietic marrow is reflected in certain definite changes in the peripheral blood picture. These changes are *reticulocytosis, macrocytosis* and *erythroblastæmia*.

Reticulocytosis in Hæmolytic Anæmia. The normal range for the reticulocyte count is usually given as between 0.2% and 2.0% in adults. In hæmolytic anæmia the proportion is often as high as 20%; occasionally the count is much higher and may reach 70% or even more (Fig. 12). Although there is no very close correlation between reticulocyte counts and the degree of anæmia, the highest counts are generally found in the more anæmic patients in whom hæmolysis is usually more intense and efforts at compensation consequently greater.

Failure of Regeneration. Occasionally the erythropoietic activity of the bone marrow may fail in the course of a chronic hæmolytic anæmia, with the result that the reticulocyte count falls to very low levels. A serious increase in anæmia may result. Dramatic examples have been described by Owren (1948), Dameshek and Bloom (1948) and Gasser (1950) in hereditary spherocytosis (see Chapter 2) and by Singer, Motulsky and Wile

TABLE 1 *Mean corpuscular volumes (M.C.V.), mean corpuscular haemoglobin concentrations (M.C.H. Conc.) and maximum reticulocyte counts in various types of congenital and acquired haemolytic anaemia*

Type of Haemolytic Anaemia	M.C.V. (c.μ)		M.C.H. Conc.		Maximum Reticulocyte Count	
	Range	Mean	Range	Mean	Range	Mean
Hereditary spherocytosis	70-99 (37)*	83.5	31-40 (18)	[illegible]	3-79 (34)	14.6
Nonspherocytic congenital haemolytic anaemia (4)	114-1[illegible]†	1[illegible]	[illegible]-34	31.6	9-70	33.8
Idiopathic acquired haemolytic anaemia (18)	87-141‡	109	28-42	31.9	1-51	19.5
Paroxysmal nocturnal haemoglobinuria (8)	87-14[illegible]	115	30-36	3[illegible].6	[illegible]-53	1[illegible].0
Normal range	76-96	86	32-36	34	0.2-2	—

The number of patients studied is indicated by the number in parentheses
* Signifies before splenectomy
† Signifies after splenectomy
‡ Some of the patients had undergone splenectomy

(1950) in sickle-cell anaemia. A chronic degree of marrow inadequacy is probably not very uncommon in paroxysmal nocturnal haemoglobinuria (Letman 1952). The reasons for acute or chronic marrow failure are poorly understood. Possibly toxic or infective processes are the main cause of acute marrow hypoplasia; the failure in erythropoiesis cannot as a rule be accounted for by deficiencies of known haemopoietic materials.

The whole question of the erythropoietic response to haemolysis has been recently considered by Crosby and Akeroyd (1952). They stressed the fact that the normal bone marrow has considerable but not unlimited powers of compensating for increased haemolysis. They calculated that the maximum possible output of haemoglobin by a healthy adult is in the region of 0.6 g. per kilo per day, at least six times that normally produced. This means, in theory at least, that haemolysis can occur at six times the normal rate, corresponding with an erythrocyte mean cell life as low as twenty days, without the patient necessarily becoming anaemic. Crosby and Akeroyd also calculated the probable haemo-

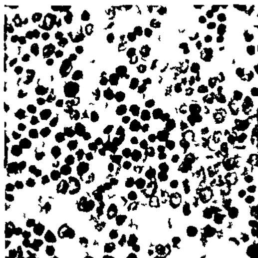


Fig 11 Photomicrograph of a section of sternal bone marrow aspirated from a patient suffering from hereditary spherocytosis The marrow is hyperplastic and normoblasts are conspicuous × 400

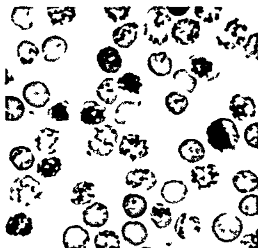


Fig 12 Photomicrograph of a blood film of a patient suffering from a congenital non spherocytic hæmolytic anæmia (Case 1 of Dacie *et al* 1953) Reticulocytes predominate Stained supravitally with brilliant cresyl blue × 1 000

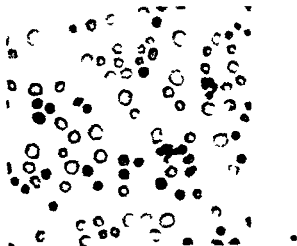


FIG. 13. Photomicrograph of a blood film of a patient suffering from an idiopathic acquired hæmolytic anæmia (Case 11). The contrast between the microspherocytes and the polychromatic macrocytes is well shown. × 400

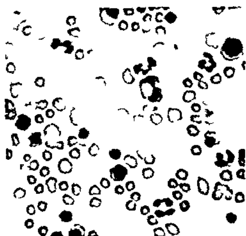


FIG. 14. Photomicrograph of a blood film of a patient suffering from an idiopathic acquired hæmolytic anæmia. Hæmolysis persisted after splenectomy. Numerous normoblasts and a single erythrophage are present.

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globin output of patients suffering from Mediterranean anæmia and pernicious anæmia and found that this was far less than 0 6 g per kilo per day

The same problem was considered by Finch and Coleman (1953) They studied the degree of erythropoietic hyperplasia in the marrow the rate of appearance of ^{59}Fe in the hæmoglobin of the peripheral blood and the morphology of the patients erythrocytes They concluded that three types of erythropoiesis could be differentiated compensated decompensated and dyserythropoiesis In the compensated type the mass of erythropoietic tissue was increased but maturation took place normally and the erythrocyte morphology was normal except for the effects of the hæmolytic process In the decompensated type the mass of erythropoietic tissue in the marrow was increased but the maturation of erythroblasts was accelerated and poikilocytes siderocytes and abnormally large numbers of reticulocytes were present in the peripheral blood In patients showing dyserythropoiesis the production of erythrocytes from the marrow fell far short of the marrow s potential capacity

Macrocytosis An increase in the average size of the erythrocytes in the peripheral blood seems to be a regular accompaniment of increased erythropoiesis whether this is a response to hæmorrhage (Lehmann 1949 Wintrobe 1951) or to hæmolysis (Dameshek and Schwartz 1940) The cause of the macrocytosis is uncertain the cells are presumably derived from unusually large precursors macronormoblasts (Dacie and White 1949) The macrocytosis is generally accompanied by an increased proportion of reticulocytes in the peripheral blood but the high proportion of reticulocytes present cannot be the whole explanation for the macrocytosis for the fully ripened corpuscles are also mostly larger than normal This increase in size is reflected in an increase in mean cell diameter as well as in an increase in mean cell volume (Fig 16) Observations on the mean cell volume of a series of patients with hæmolytic anæmia are given in Table 1 Where *there is conspicuous spherocytosis the contrast between the* macrocytic reticulocytes and spherocytic fully ripened corpuscles is often most striking (Fig 13)

Erythroblastæmia Normoblasts are not infrequently present in the peripheral blood stream of patients with hæmolytic anæmia Usually however, there are less than 1 per 100 leucocytes In general the higher the reticulocyte count and the more anæmic the patient the more frequent are the normoblasts In young children, however, erythroblastæmia may be a well marked feature

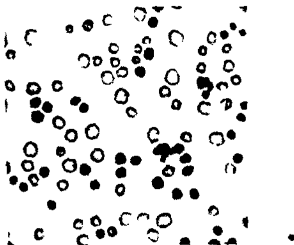


Fig. 13. Photomicrograph of a blood film of a patient suffering from an idiopathic acquired hæmolytic anæmia (Case 11). The contrast between the microspherocytes and the polychromatic macrocytes is well shown. × 400

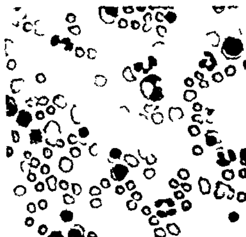


Fig. 14. Photomicrograph of a blood film of a patient suffering from an idiopathic acquired hæmolytic anæmia. Hæmolysis persisted after splenectomy. Numerous *normoblasts* and a single erythrophage are present. × 400

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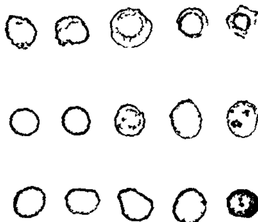


FIG 15 Photomicrographs of normoblasts and erythrocytes stained by Perls's reaction to show siderotic granules (top two rows) and stained by Jenner Giemsa stain to demonstrate Pappenheimer bodies (bottom row) (From Douglas and Dacie 1953)

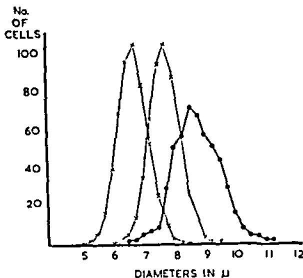


Fig 16 Erythrocyte-diameter distribution curve (Price-Jones curve) made from a dried peripheral blood film of a patient suffering from a congenital non-spherocytic hæmolytic anæmia (Case 1 of Dacie *et al* 1953) M C D 8 7 μ σ = 0 77 μ The thin outlines indicate the maximum and minimum normal curves

in many types of hæmolytic anæmia particularly is this so in hæmolytic disease of the newborn In adults the presence of large numbers of normoblasts should cause the observer to reconsider the diagnosis of a primary hæmolytic anæmia However it is not uncommon to find numerous normoblasts in the peripheral blood of patients suffering from acquired hæmolytic anæmia of the auto antibody type in whom hæmolysis has persisted at a rapid rate following splenectomy (Fig 14)

Siderocytosis

Siderocytes are erythrocytes containing granules giving Perls s Prussian blue reaction for ionized ferric iron They were observed by Gruneberg (1941a and b) in small numbers in normal rat mouse and human embryos and in large numbers in mice with a congenital anæmia (Gruneberg 1942) They were first recognized in adult human blood by Doniach Gruneberg and Pearson (1943) It is now realized that siderocytes may be found in the peripheral blood in a wide range of blood disorders particularly after splenectomy (Douglas and Dacie 1953) Siderotic granules at

any rate those demonstrated by the simple HCl potassium ferro cyanide method are not indicative of a hæmolytic process nor does their presence indicate a dying cell (cf Case 1945) on the contrary the granules appear in developing erythroblasts at the same time as hæmoglobin is being formed (Gruneberg 1942 Dacie and Doniach 1947) The siderotic granules stain also with Romanowsky dyes and when stained in this way have been referred to by McFadzean and Davis (1947) as ' Pappenheimer bodies (Pappenheimer Thompson Parker and Smith 1945) (Fig 15)

The incidence and significance of siderocytes have been recently assessed by Douglas and Dacie (1953) Their findings may be summarized as follows Iron containing granules may be recog nized in a large proportion of the normoblasts of normal bone marrow a small number of normal marrow reticulocytes are siderocytes but few if any siderocytes can be found in the peri pheral blood of normal subjects The proportion of normoblasts containing iron granules is increased and the granules may be unusually large in diseases where there is a defect in hæmoglobin synthesis or erythropoiesis In iron deficiency on the other hand iron containing granules are absent from the normoblasts The presence of iron containing granules in normoblasts is thus a normal phenomenon It appears that more iron is taken into the erythroblast during hæmoglobin synthesis than can be immediately incorporated into hæm and that this excess iron may be utilized during the later stages of normoblast ripening and during the early reticulocyte stage The exact nature of the stainable iron needs elucidation

Siderocytes are seldom seen in the peripheral blood in patients with blood diseases except after splenectomy when they may often be found in very large numbers It seems possible that in the absence of the spleen the rate of maturation of siderotic granules is delayed thus causing the siderocytes to appear in the peripheral blood Splenectomy does not appear to cause any increase in the numbers of erythroblasts containing iron granules in the marrow nor could Douglas and Dacie demonstrate that the spleen filtered off siderocytes from the circulation as has been claimed (McFadzean and Davis 1949 Pirrie 1952) Douglas and Dacie found the highest siderocyte counts in the peripheral blood of patients with high reticulocyte counts which had per sisted after splenectomy or in patients suffering from defects in hæmoglobin synthesis who had undergone splenectomy (Fig 56 p 121)

TABLE 2 *Incidence of erythrocytes and normoblasts containing iron demonstrable by Perls's reaction in the peripheral blood and bone marrow of normal subjects and patients suffering from various hæmolytic disorders (abridged from Douglas and Dacie 1953)*

Type of Case	Peripheral Blood			Bone Marrow				
	Number of Patients	Siderocytes		Number of Patients	% Siderocytes		% Iron-containing Normoblasts	
		Range	Mean		Range	Mean	Range	Mean
Normal	19	0	0	18	0–3	0·5	24–81	49
Probably normal erythropoiesis (after splenectomy)	11	0–14	4·0	3	0–1·5	0·7	11–34	26
Hereditary spherocytosis	17	0–[illegible]	0·[illegible]	7	0·0	0·0	12–87	43
(after splenectomy)	16	2–45	10	2	3–30	17	[illegible]	4[illegible]
Atypical congenital hæmolytic anæmia	6	0–6	1·0	4	1–11	4·8	21–[illegible]	3
(after splenectomy)	11	3[illegible]–83	[illegible]9	9	0–[illegible]	5·2	20–77	47
Mediterranean anæmia	4	0	0	3	0–[illegible]	1·0	21–55	41
Sickle cell anæmia	1	0·2	—	1	0·0	—	44	—
Acquired hæmolytic anæmia	19	0–21	2·3	9	0–[illegible]	3·2	4–78	37
(after splenectomy)	13	1–67	20	2	7–10	13	30–51	41
Hæmolytic disease of the newborn	14	0–35	3·7	—	—	—	—	—

Crosby (1953) has suggested that the spleen in some way removes the siderotic granules from erythrocytes without actually destroying the cells He transfused blood containing many siderocytes obtained from a patient who had undergone splenectomy into a recipient who had a normal spleen and found that the siderotic granules disappeared within three hours although the transfused cells were not destroyed Whether the spleen actually removes the granules as suggested by Crosby or in some way accelerates their metabolism within the cells remains to be seen

The incidence of siderocytes in different types of hæmolytic anæmia before and after splenectomy is illustrated in Table 2

SPECIAL LABORATORY TESTS USEFUL IN INVESTIGATING THE HÆMOLYTIC ANÆMIAS

The tests to be described in this section are the *osmotic* and *mechanical* fragility tests and certain serological procedures They will be discussed in general terms only with particular reference to their significance in diagnosis Further details will be given later when the various types of hæmolytic anæmia are described Technical details are given in Chapter 18

Osmotic Fragility

The introduction of the fragility test into clinical laboratory practice seems to have quickly followed the pioneer observations of Chauffard (1907) on the decreased resistance to hypotonic salt solution of the erythrocytes in l ictère congenital de l adulte (hereditary spherocytosis) Although the correlation between a reduction in the diameters of mammalian erythrocytes and increase in osmotic fragility had been demonstrated by Vallery Radot and Lhéritier in 1919 it was not until Haden s (1934) paper that increased osmotic fragility was satisfactorily correlated with spherocytosis Other confirmatory publications followed (Castle and Daland 1937 Dacie and Vaughan 1938) and it is generally held to day that erythrocyte shape is a major factor in determining osmotic fragility

It is also quite clear that increased osmotic fragility is not the monopoly of any particular type of hæmolytic anæmia Definite increases in fragility are found in hereditary spherocytosis (almost invariably) in idiopathic acquired hæmolytic anæmia (most patients) in hæmolytic disease of the newborn due to anti A (less commonly in sensitization due to anti Rh) in hæmolytic anæmia due to chemical poisoning and in severe burning etc i e in just

those cases in which spherocytosis is usually obvious in blood films However it should be added that sometimes deviations from the normal are slight and that a carefully standardized technique is required to detect them Osmotic fragility is normal in most cases of secondary or symptomatic hæmolytic anæmia, and in paroxysmal nocturnal hæmoglobinuria In Mediterranean anæmia sickle-cell anæmia and in other allied disorders there is characteristically an increased resistance to hæmolysis with or without a small proportion of unusually fragile cells

A number of variants of the osmotic fragility test have been introduced from time to time Some of the variations are technical ones and are concerned with such things as the way in which hæmolysis is measured and the proportion of blood added to saline Other variations have involved the use of unusual hæmolysing solutions for instance Dickstein and co workers (1949) employed, in addition to simple hypotonic saline solutions of glycerine and thiourea in hypotonic saline The important thing is for the test to be carried out in as completely a standardized way as possible

Recording the Results of Osmotic Fragility Tests Most workers have not been content to record merely the highest concentration of saline in which hæmolysis is just detectable (initial lysis or minimum resistance) and the highest concentration of saline in which hæmolysis appears to be complete (complete lysis or maximum resistance) It is advantageous at least to record in addition the concentration of saline causing 50% lysis (median corpuscular fragility (M C F) Vaughan 1947 Dacie and Vaughan 1938) It is worth while too when a range of hypotonic solutions has been used to construct a fragility curve by plotting on graph paper the percentage of hæmolysis in each tube against the corresponding concentration of salt solution In normal subjects an almost symmetrical curve of sigmoid shape is obtained (Fig 17) In disease however deviations from the normal type of curve are found the curves for instance may have long tails due to the presence of a small proportion of very fragile cells or intermediate forms may be found The tailed type of curve is commonly found in cases of hereditary spherocytosis before splenectomy (Dacie 1943)

Two other simple alternative methods of recording the results quantitatively are available the data may be plotted on probability paper (Hunter 1939 Parpart *et al* 1947 Crawford Cutbush and Mollison 1953) or increment hæmolysis curves can be drawn (see below) Both methods emphasize any heterogeneity in the osmotic fragility of the cell population should this

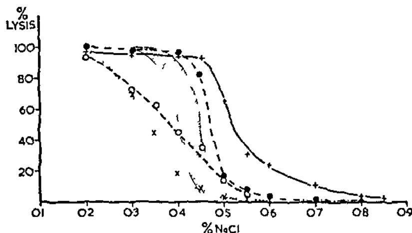


Fig 17 Osmotic fragility curves of patients suffering from (a) sickle cell anæmia x ——— x (b) Mediterranean anæmia o ——— o (c) hereditary spherocytosis ● — — — ● and (d) idiopathic acquired hæmolytic anæmia (warm auto antibody type) x——x The shaded area represents the normal range

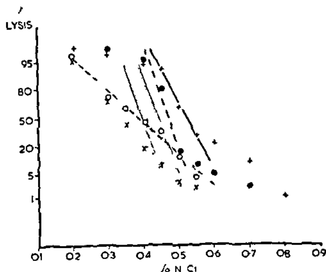


Fig 18 As Fig 17 except that the observations on osmotic fragility are plotted on arithmetical probability paper (same symbols as in Fig 17)

be present If the observed amounts of hæmolysis are plotted on the probability scale against concentrations of saline an almost straight line can be drawn though the points in the case of normal blood there being skewness only where hæmolysis is becoming almost complete (Fig 18) This method enables the M C F to be read off with ease In disease tailed curves result in varying degrees of skewness at the other end of the probability plot as well (Crawford *et al* 1953) (Fig 18) Increment hæmolysis curves

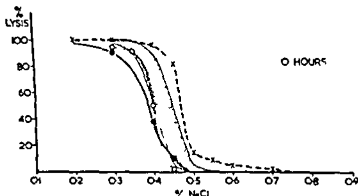


FIG 19*a*

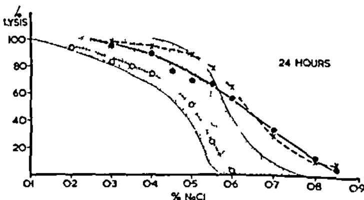


FIG 19*b*

FIG 19 Osmotic fragility curves before and after incubating at 37 C for 24 hours the blood of patients suffering from (*a*) hereditary spherocytosis x x (*b*) •———• and (*c*) o o congenital non-spherocytic hæmolytic anæmia (Cases 1 and 3 of Dacie *et al* 1953 respectively) The shaded areas represent the normal range

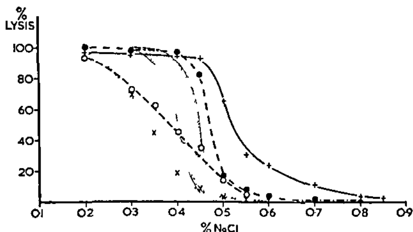


FIG 17 Osmotic fragility curves of patients suffering from (*a*) sickle cell anæmia x x (*b*) Mediterranean anæmia o o (*c*) hereditary spherocytosis ● — — — ● and (*d*) idiopathic acquired hæmolytic anæmia (warm auto antibody type) x——x The shaded area represents the normal range

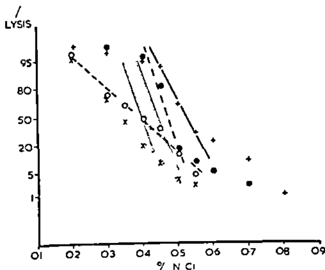


FIG 18 As Fig 17 except that the observations on osmotic fragility are plotted on arithmetical probability paper (same symbols as in Fig 17)

The rate of autohæmolysis is conveniently studied using defibrinated blood (see p 479) However the essential differences between the behaviour of normal and pathological erythrocytes are not altered in the presence of anticoagulants (Dacie 1941)

Spherocytosis is not the only cause of an accelerated spontaneous lysis of blood kept under sterile conditions *in vitro* In

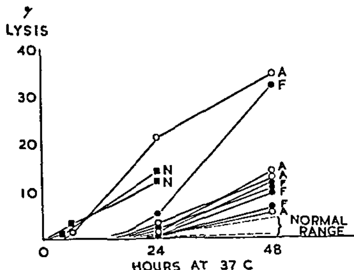


FIG 20 Spontaneous hæmolysis resulting from the incubation at 37 C of the sterile defibrinated blood of patients suffering from (A) acquired hæmolytic anæmia of the auto antibody type (F) hereditary spherocytosis and (N) paroxysmal nocturnal hæmoglobinuria (from Dacie 1950a)

poisoning with hæmotoxic chemicals such as acetylphenylhydrazine the same phenomenon may be observed (see p 387) and this is also true of paroxysmal nocturnal hæmoglobinuria In the latter disease spontaneous hæmolysis *in vitro* is most characteristic and occurs rapidly major degrees of lysis often being visible within an hour or so It is best observed in clotted blood—hæmoglobin will be seen to diffuse from the clot into the surrounding serum—rather than in defibrinated blood or blood to which anticoagulants have been added In defibrinated blood lysis is largely inhibited by loss of carbon dioxide and consequent rise in pH and anticoagulants if in sufficient concentration may inhibit the hæmolytic reaction entirely (see p 424)

have been drawn by Momigliano and Bairati (1935) Suess *et al* (1948) and by Bolton (1949) With this method the differences in hæmolysis between adjacent tubes are plotted against the corresponding saline concentrations definitely bimodal curves may be obtained for instance during recovery from a hæmolytic episode

Osmotic Fragility after Incubation Recently certain workers have estimated the osmotic fragility of the erythrocytes after the blood has been incubated for twenty four hours at 37 C (Young 1947 Emerson Shen Ham and Castle 1947 Varadi 1951 Dacie *et al* 1953 Selwyn and Dacie 1954) Under these circumstances the erythrocytes from patients with hereditary spherocytosis generally undergo a greater increase in fragility than do normal cells By this procedure it may be possible to differentiate more clearly between patients with hereditary spherocytosis of the mildest degree and normal subjects Dacie and his co workers (1952) found too that in certain non spherocytic congenital hæmolytic anæmias fragility might increase by an abnormal amount as the result of incubation despite the fact that the fragility test carried out before incubation gave a normal result (Fig 19) The changes following incubation in other types of hæmolytic anæmia have been less thoroughly studied

The extent of the increase in osmotic fragility brought about by incubation in most cases runs parallel with the amount of spontaneous lysis which results from incubation (Selwyn and Dacie 1954)

Autohæmolysis

When normal defibrinated blood is incubated under sterile conditions at 37 C little or no lysis takes place in the first forty eight hours thereafter autohæmolysis develops quite rapidly The exact sequences of changes which precede lysis are not yet fully known It is known however that if the glucose concentration is maintained the rate of hæmolysis is usually significantly slowed (Selwyn and Dacie 1954) Dacie (1941) showed that in cases of hereditary spherocytosis the rate of autohæmolysis was significantly increased and might take place at five to ten times the normal rate He found too that there was a general correlation between the osmotic fragility (of unincubated blood) and the subsequent rate of lysis on incubation the most fragile bloods undergoing the most rapid lysis Blood from patients with acquired hæmolytic anæmia and spherocytosis may similarly undergo relatively rapid autohæmolysis (Dacie 1950a) (Fig 20)

The mechanical fragility test although it provides interesting information seems hardly likely to be used as a routine laboratory method The actual technique needs careful standardization and the fact that several different types of erythrocyte abnormality lead to an increased susceptibility to mechanical trauma reduces its diagnostic value

SEROLOGICAL TESTS

In the acquired hæmolytic anæmias the demonstration of abnormal antibodies on the surface of the patient s erythrocytes or in his serum has proved to be of great diagnostic importance Only a brief general account of the methods of demonstrating the antibodies will be undertaken at this stage Further details are given in Chapters 9 and 18

The Direct Antiglobulin Reaction (Coombs s Test)

When auto antibodies are playing a part in bringing about erythrocyte destruction *in vivo* it is probable that the patients washed erythrocytes will always be found to be agglutinated by an anti human globulin serum i e the direct antiglobulin (Coombs) test will be positive This test was introduced by Coombs Mourant and Race (1945) as a method for detecting incomplete Rh antibodies The same principle had been employed many years previously by Moreschi (1908) who demonstrated that erythrocytes sensitized with heterologous sera could be agglutinated by antibodies formed against the heterologous protein This work however had been forgotten The test as re introduced by Coombs Mourant and Race detects incomplete antibodies which lack the property of causing direct agglutination *in vitro* If cells which have adsorbed this type of antibody are washed in several changes of saline so as to free them from surrounding plasma or serum then they will be found to be agglutinated if subsequently suspended in an anti human globulin serum (Coombs s serum) This can be conveniently made by immunizing a rabbit against human serum (see Chapter 18) The antiglobulin test has proved to be a very sensitive one it is capable of detecting incomplete antibodies of many different types including those of acquired hæmolytic anæmia

The sensitivity of the method however creates pitfalls in interpretation It cannot be assumed for instance that a positive direct antiglobulin test necessarily indicates that the patient is suffering from auto immune hæmolytic anæmia Excluding false

Thus an accelerated rate of spontaneous hæmolysis may be due to several causes amongst them spherocytosis the effects of certain chemicals and the erythrocyte abnormality of paroxysmal nocturnal hæmoglobinuria The phenomenon is clearly a non specific one If hæmolysis is accelerated the observer is entitled to consider it as a valuable pointer to a hæmolytic process but nothing more The heat resistance test of Hegglin and Maier (1944) is discussed on p 433 in connection with the diagnosis of par oxysmal nocturnal hæmoglobinuria

Lysolecithin Fragility

The measurement of the resistance of human erythrocytes to solutions of lysolecithin was used by Singer (1937 1940) and by Gripwall (1948) in the investigation of cases of hæmolytic anæmia Singer and Gripwall found that whereas the resistance to lysoleci thin was definitely diminished in cases of hereditary spherocytosis it was normal in symptomatic types of hæmolytic anæmia even though osmotic resistance was diminished The test has not been widely applied The diminished resistance of hereditary sphero cytes was confirmed by Maier (1947) who however observed reduced lysolecithin resistance in a patient with splenic vein thrombosis in whom osmotic fragility was almost normal Foy and Kondi (1943) found that the resistance to lysolecithin of the erythrocytes of a patient suffering from blackwater fever was diminished although the osmotic fragility was normal The significance of these observations is not known If the test really is capable of differentiating clearly between hereditary and acquired spherocytes it deserves to be more widely used

Mechanical Fragility

Normal erythrocytes are susceptible to mechanical trauma and may be readily lysed *in vitro* by shaking with glass beads Increased susceptibility to lysis has been observed in certain pathological states and if the test is performed quantitatively it can be used in the investigation of hæmatological disorders (Shen Castle and Fleming 1944 Young Izzo and Platzer 1951 Dacie *et al* 1953)

Spherocytes sickled cells and agglutinated corpuscles have been shown to have an increased susceptibility to mechanical trauma but poikilocytes do not seem to be especially fragile unless spherocytic Goldbloom Fischer Reinhold and Hsia (1953) have recently reported that the mechanical fragility of newborn infants is almost double that of older children or adults

give an optimum reaction with say erythrocytes sensitized with anti D will react equally well with corpuscles sensitized with other antibodies Cold antibodies for instance react best in high concentrations of a potent antiglobulin serum and may fail to react in serum diluted 1 in 64 or 1 in 128 concentrations which nevertheless may cause maximum agglutination of corpuscles sensitized with anti D For this reason it is always wise to carry out the antiglobulin test using a range of dilutions of the rabbit anti human globulin serum

Other points concerning the antiglobulin reaction such as the effects of acidification and heat inactivation of the patients sera and of the temperature at which sensitization is carried out and the way in which the test helps in the differentiation of different types of antibody are dealt with in Chapters 9 and 18

Detection of Antibodies in Patients Sera

Various methods are available and an outline only will be attempted here (Further details are given in Chapters 9 and 18)

Spontaneous auto agglutination occurring at room temperature or at 37 C is a pointer to the presence of abnormal antibodies

Non specific *complete* (in saline agglutinating) antibodies may be titrated using normal corpuscles of the same blood group as the patient or normal group O corpuscles It is useful to carry out such titrations at various temperatures between 2 C and 37 C as most complete antibodies found in the sera of patients with hæmolytic anæmia excluding immune iso antibodies are cold ones

Hæmolytic antibodies can be detected in certain sera using either normal corpuscles in patients sera acidified to between pH 6 5 and 7 0 (except with antibodies of the Donath Landsteiner type which react best in unacidified serum) or by the use of trypsinized normal (T.N) erythrocytes or paroxysmal nocturnal hæmoglobinuria (P N H) erythrocytes As the majority of hæmolytic antibodies are of the cold variety sensitization should be carried out at 20 C as well as at 37 C

Incomplete antibodies can be detected in at least three ways by means of the indirect antiglobulin (Coombs) test the sensitizations being carried out at the appropriate temperature and pH for the antibody under investigation by the use of trypsinized normal corpuscles or corpuscles acted upon by other proteolytic enzymes such as papain and by titration in an albumin medium rather than in saline The first two techniques are particularly useful Both should be carried out as the results obtained are

positive tests due to the use of inadequately absorbed antiglobulin sera (see p 496) positive tests may occasionally be given by the blood of patients suffering from a variety of diseases or even by that of a normal subject

One type of positive reaction is due to sensitization occurring *in vitro* If for instance clotted or defibrinated normal blood is allowed to stand in a refrigerator at 0 to 2 C and the antiglobulin test subsequently carried out on corpuscles obtained from the chilled blood the reaction may be positive due to adsorption of incomplete cold antibodies normally present in human sera (Dacie 1950b) Corpuscles obtained from chilled oxalated or heparinized blood are less likely to give this type of positive reaction as the presence of anticoagulants inhibits adsorption of the antibody Sensitization by cold antibodies is not however the only cause of unexpected positive antiglobulin reactions In some instances the reaction will be found to be positive even if the possibility of chilling *in vitro* has been excluded by collecting the patient s blood directly into saline warmed at 37 C The cause of this type of ' non specific reaction has not as yet been determined It must be a very rare event with blood from a strictly healthy person it is not however uncommon in diseases such as rheumatoid arthritis disseminated lupus erythematosus, leukæmia myelosclerosis sarcoid and aplastic anæmia conditions in which abnormal amounts of globulins are often found in the serum Nevertheless it does not seem to be possible to correlate the incidence of positive reactions with the presence of abnormal amounts of any particular type of globulin In particular the reaction is usually negative in cases of hepatic cirrhosis and multiple myeloma despite great increases in gamma globulins

The non specific reactions referred to in the preceding paragraph are usually weak ones they are maximal in high concentrations of a potent antiglobulin serum The reactions are relatively insensitive to the addition to the antiglobulin serum of small amounts of human γ globulin a feature which distinguishes them from the reactions of most of the warm antibodies found in cases of auto immune acquired hæmolytic anæmia (see p 235)

Falsely negative reactions may be due to three main causes the antiglobulin serum may be relatively impotent and only capable of detecting strongly sensitized corpuscles the corpuscles to be tested may have been insufficiently washed free from surrounding plasma or serum and the antiglobulin serum may have been used at an inappropriate dilution It is wrong to suppose that the dilution of an antiglobulin serum which will

quoted anti A or anti M respectively—then the unagglutinated cells will be very largely those of the donor If known dilutions of blood are made in the agglutinating serum and if the procedure is carried out carefully and in a standard way then the actual numbers of unagglutinated cells per c mm of blood may be estimated quite accurately

Ashby's method was not widely applied until the Second World War Then it was employed as a means of assessing the relative value of anticoagulant solutions in the preservation of blood (Mollison and Young 1940 1942) Dacie and Mollison (1943) modified the technique by introducing the idea of centrifuging the erythrocyte suspensions in the agglutinating serum and thus enhancing agglutination They applied the method to the study of six patients with hereditary spherocytosis in five of the patients the transfused normal blood survived for a normal length of time i e 100 to 120 days in the sixth patient elimination was complete in 60 days—this patient who was Rh negative was subsequently found to have been given inadvertently Rh positive blood The blood of one of these patients was transfused to a normal recipient As already referred to (p 11) in striking contrast to the normal survival of normal blood transfused to the patients this patient's blood survived both before and after the patient had undergone splenectomy for only a short time in the normal recipient Loutit and Mollison (1946) and Mollison (1947) reported observation on patients suffering from various types of acquired hæmolytic anæmia they showed conclusively in complete contrast to the results obtained in hereditary spherocytosis that these latter patients might eliminate transfused normal erythrocytes extremely rapidly

The general accuracy of the results of these early transfusion experiments has been confirmed subsequently in many centres of the world It has been found too that in other hereditary hæmolytic anæmias also due apparently to intrinsic corpuscular defects normal corpuscles survive normally in the patients whilst the patients corpuscles are more or less rapidly eliminated when transfused to normal recipients This has been shown to be so for instance in sickle-cell anæmia (see p 155) and in elliptocytic and other atypical congenital hæmolytic anæmias (Crosby 1950 Dacie *et al* 1953) Similar results have been obtained in paroxysmal nocturnal hæmoglobinuria (see p 422)

In all the disorders referred to in the preceding paragraph the rate of elimination of the normal corpuscles from the recipient's circulation has been slow and uniform about 1% or a little less

complementary some antibodies being better detected by one technique than by the other and *vice versa* The results obtained by the albumin method usually parallel those obtained by the indirect antiglobulin reaction

As will be referred to in later chapters patients suffering from acquired hæmolytic anæmia not uncommonly develop immune iso antibodies following transfusions These have to be taken into account in investigating the patient s serum for non specific antibodies Moreover recent work suggests that some of the auto antibodies developed by patients have definite specificities (see Chapter 9) In the detection and accurate characterization of a patient s antibodies it is desirable to have available therefore a large panel of normal bloods of known genotype with which to test his serum or eluates made from his erythrocytes An important preliminary step is to determine the patient s own genotype before he receives any transfusions

Other Serological Tests

The most important test relevant to the diagnosis of hæmolytic anæmia that has not yet been mentioned is the acidified serum test (Ham s test) used in the diagnosis of paroxysmal nocturna hæmoglobinuria (P N H) The essence of this simple test is to see whether the patient s corpuscles undergo rapid hæmolysis a 37 C in normal serum acidified to a pH between 6 5 and 7 0 When carried out with certain essential controls a positive tes appears to be specific for the P N H erythrocyte abnormality

THE ESTIMATION OF THE LIFE SPAN OF ERYTHROCYTES AS A METHOD OF INVESTIGATING HÆMOLYTIC ANÆMIAS

Ashby s Method The differential agglutination method introduced by Ashby (1919) as a means of studying the fate and survival of the erythrocytes of one subject after transfusion int another has proved a valuable tool in the investigation of th hæmolytic anæmias Briefly the method consists of the trans fusion into the circulation of the recipient of erythrocytes whic are compatible but which are nevertheless of a serologicall different group For example group O corpuscles might b transfused to a group A or AB recipient and group ON corpuscle into a group OM or OMN recipient If blood taken from th recipient after transfusion is then suspended in a potent aggli tinating serum active against the recipient s cells—in the example

quoted anti A or anti M respectively—then the unagglutinated cells will be very largely those of the donor If known dilutions of blood are made in the agglutinating serum and if the procedure is carried out carefully and in a standard way then the actual numbers of unagglutinated cells per c mm of blood may be estimated quite accurately

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In all the disorders referred to in the preceding paragraph the rate of elimination of the normal corpuscles from the recipient's circulation has been slow and uniform about 1% or a little less

of the corpuscles disappearing every day When the results are plotted on graph paper the course of elimination is almost straight, i e linear (Fig 21) This is consistent with the idea that the life span of normal erythrocytes is comparatively constant and that elimination from the circulation in health is a function of age alone In the acquired hæmolytic anæmias not only is the rate of elimination greatly accelerated but the course of elimination is

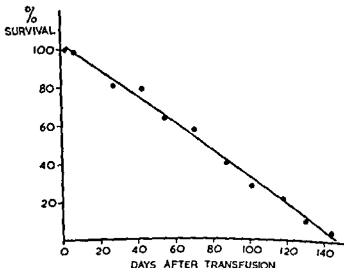


FIG 21 Survival of normal corpuscles transfused to a patient suffering from a congenital non spherocytic hæmolytic anæmia (Case 1 of Dacie *et al* 1953) The normal corpuscles were eliminated in a linear fashion and survived well over 120 days

also different when plotted on graph paper the disappearance of the corpuscles is at first rapid and then gradually slows (Fig 22) This curvilinear type of elimination was referred to as exponential by Brown Hayward Powell and Witts (1944) It has generally been interpreted as being due to a random form of destruction in which the age of the corpuscles is unimportant The difficulties of exact interpretation and the complexities which result when attempts are made to analyse the form of elimination curve mathematically are illustrated in the papers of Brown *et al* (1944) Callender Powell and Witts (1947) Dornhorst (1951) Sheets Janney Hamilton and deCowin (1951) Evans Amatuzio and Ebert (1952) and Eadie and Brown (1953)

Recording Results of Transfusion Studies There are various ways in which the data obtained by means of a survival

study may be expressed: the *end point* of elimination or the *half life* (the time at which 50% of the transfused erythrocytes have been eliminated) may be recorded or the *mean cell life* calculated.

The *end point* of elimination is difficult to determine with accuracy unless a very large transfusion has been given. However

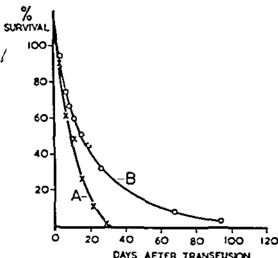


FIG 22. Survival curves plotted on ordinary graph paper of normal corpuscles transfused to a patient (Case 13) suffering from idiopathic acquired hæmolytic anæmia of the cold antibody type. (A) Survival before splenectomy. (B) survival after splenectomy.

It is possible to calculate the mean cell life from a knowledge of the area under the elimination curve if hæmolysis is complete in 30 days or less (Dornhorst 1951). Applying the calculation to curve A:

The total area = 277 square graph paper units
Initial height = 50 units
$$\frac{\text{Area}}{\text{Height}} = 5.5 \text{ units}$$
5.5 units on graph paper = 11 days
mean cell life = 11 days

when the plot of the elimination results takes the form of a straight line it is permissible to extend this to cross the time axis (abscissa) and to take this as representing the end point. Even so some inaccuracy is inevitable as the termination of the elimination plot is probably normally curved due to variation in the normal life span of the cell population (Dornhorst 1951). A simple alternative is to determine the *half life* of the transfused corpuscles by

of the corpuscles disappearing every day When the results are plotted on graph paper the course of elimination is almost straight i e linear (Fig 21) This is consistent with the idea that the life span of normal erythrocytes is comparatively constant and that elimination from the circulation in health is a function of age alone In the acquired hæmolytic anæmias not only is the rate of elimination greatly accelerated but the course of elimination is

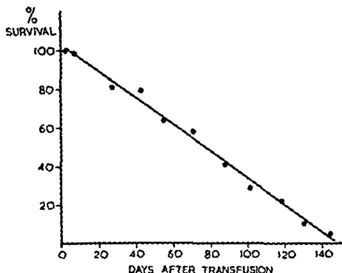


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also different when plotted on graph paper the disappearance of the corpuscles is at first rapid and then gradually slows (Fig 22) This curvilinear type of elimination was referred to as exponential by Brown Hayward Powell and Witts (1944) It has generally been interpreted as being due to a random form of destruction in which the age of the corpuscles is unimportant The difficulties of exact interpretation and the complexities which result when attempts are made to analyse the form of elimination curve mathematically are illustrated in the papers of Brown *et al* (1944) Callender Powell and Witts (1947) Dornhorst (1951) Sheets Janney Hamilton and deGowin (1951) Evans Amatuzio and Ebert (1952) and Eadie and Brown (1953)

Recording Results of Transfusion Studies There are various ways in which the data obtained by means of a survival

corpuscles remain undestroyed (Dornhorst 1951) (Fig 23) Alternatively when the rate of elimination is fast enough for the effect of ageing to be unimportant (e g the elimination is completed in less than 30 days) the mean cell life can be estimated by dividing the area covered by the survival curve drawn on ordinary arithmetical graph paper by the initial height of the curve (Dornhorst 1951) (Fig 22) In experiments in which congenitally defective corpuscles are transfused to normal recipients the mean cell life may be obtained by continuing as a straight line the initial steep part of the survival curve This line will cut the time axis at approximately the mean cell life (Mollison 1951 Crosby and Akeroyd 1952)

Modifications of Ashby s Method A sedimentation differential agglutination test has been evolved Instead of counting the numbers of unagglutinated cells after transfusion Stats (1950) measured the height of the sedimented agglutinated cells after the mixture of donor s and recipient s cells had been allowed to stand in a sedimentation tube The proportion of agglutinated cells could be calculated from the height of the column of the sedimented cells using a calibration graph made for each agglutinating serum This method can be applied in exchange transfusions to determine approximately the proportion of agglutinable recipient s cells remaining A minor modification of the Ashby method was introduced by Hurley and Weisman (1953) who deliberately used hæmolytic anti A or anti B sera They claimed that the counting of the unaffected donor cells was facilitated by the removal of the recipient s cells by lysis rather than by agglutination

Methods Employing Isotopes

^{15}N Shemin and Rittenburg (1945) using glycine labelled with ^{15}N demonstrated that the amino acid was used in the formation of protoporphyrin from which hæmoglobin was derived Later they were able to show that if glycine labelled with ^{15}N was ingested it was possible to calculate the mean life span of the erythrocytes by analysis of the isotope-concentration/time curve of hæmin (Shemin and Rittenburg 1947) A figure of 127 days for the life span was obtained in one normal subject London Shemin West and Rittenburg (1949) subsequently studied the ^{15}N concentration/time curves of hæmin in patients suffering from several different blood disorders In a patient with sickle-cell anæmia the half life of the erythrocytes was calculated to be twenty nine days and in a patient with pernicious anæmia the

reading from the graph of elimination the time at which 50% of the transfused corpuscles have disappeared It is probably preferable however to calculate the *mean cell life* if attempts are made to correlate rates of cell destruction with for instance, pigment excretion (Crosby and Akeroyd 1952) When normal blood has been transfused to a subject with an abnormal hæmo

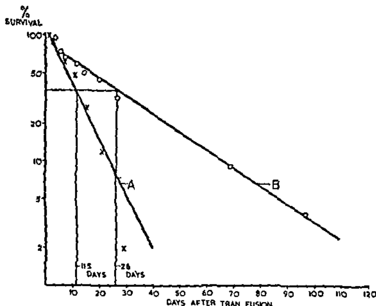


FIG 23 Survival of normal corpuscles transfused to Case 13 plotted on semi logarithmic paper (A) Before splenectomy (B) after splenectomy [Same data as in Fig 22] Straight lines have been fitted to the data and perpendiculars dropped corresponding with the survival of 37% of the transfused corpuscles The perpendiculars cut the time axis at the mean cell life (Dornhorst 1951)

Mean cell life (A) = 11.5 days (cf Fig 22)
(B) = 26 days

lytic mechanism either of two simple methods can be used to calculate the mean cell life In cases where the elimination occurs in a random fashion irrespective of the age of the donor cells the mean cell life can be ascertained by plotting the numbers of erythrocytes surviving on a logarithmic scale against time on a linear scale A straight line should fit the experimental observations the mean cell life can be obtained by dropping a perpendicular to the time axis from the point where 37% of the transfused

1953 Necheles Weinstein and LeRoy 1953 Weinstein and LeRoy 1953) The chromium is thought not to impair the viability of the cells exposed to it However slow elution takes place and has to be allowed for in calculating cell survival This method seems to be a promising one—particularly in studying the immediate post transfusion survival of either freshly-drawn or stored blood The method has the great advantage over the Ashby technique that a patient s corpuscles can be studied in his own vascular system and that the risk of transmitting virus hepatitis from one person to another is eliminated (Ebaugh Emerson and Ross 1953)

^{14}C. Radio carbon has also been used in studies of erythrocyte survival Berlin Lawrence and Lee (1951) administered glycine tagged with ^{14}C to several patients suffering from chronic leukæmia or polycythæmia The ^{14}C concentration/time curve for hæmin closely resembled that obtained using ^{15}N There was evidence of an increased rate of hæmolysis in all but one of the patients studied

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mean cell life was estimated to be 85 days before treatment and 129 days after treatment These results are of great interest and have the advantage of being studies of the patients own corpuscles in their normal environment However the method needs complex and expensive apparatus and materials and the data for erythrocyte survival is not likely to be more accurate than can be obtained by the eminently practical Ashby method On the other hand the ^{15}N method has the advantage of being capable of providing at the same time valuable information on bile pigment metabolism as well as on the life span of the erythrocytes

Erythrocytes previously tagged with ^{15}N in one subject have been transfused to another recipient (Watson James *et al* 1953) This type of experiment also gives simultaneous information on the disappearance of the tagged erythrocytes and the appearance of tagged stercobilin in the fæces In one experiment ^{15}N was first observed in the fæces of the recipient seventy days after the ingestion of ^{15}N containing glycine by the donor The concentration of ^{15}N in the stercobilin reached its peak on the 122nd day

^{59}Fe One or other of two isotopes of iron ^{55}Fe or ^{59}Fe has been used in determining the survival of erythrocytes after transfusion (Ross and Chapin 1943 Gibson *et al* 1947) The first step in the method is to give to the donor a small amount of radio iron usually ^{59}Fe with a relatively short half life (47 days) This radio iron is incorporated in newly synthesized hæmoglobin The donor is bled and the recipient transfused when a sufficient time has elapsed for the donor s peripheral blood to have acquired the required degree of isotope activity (the maximum concentration is attained about twenty one days after the administration of the radio iron) The decline in the radio activity of the recipient s blood can then be measured This technique suffers from the disadvantage that radio iron liberated from destroyed transfused erythrocytes is more or less quantitatively re utilized for the synthesis of fresh hæmoglobin The method is thus useless in determining the end point of elimination of the transfused corpuscles in cases where compensatory erythropoiesis is active On the other hand it gives valuable results when lysis takes place very rapidly e g in determining the immediate survival of stored blood (Gibson *et al* 1947)

^{51}Cr Sodium chromate tagged with ^{51}Cr has been recently used in the investigation of erythrocyte survival The corpuscles of one person can be transfused after tagging to another recipient or back into the donor himself (Ebaugh Emerson and Ross

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In other types as for instance in hereditary spherocytosis the hæmoglobin itself is normal and the abnormality seems to depend on a subtle defect in erythrocyte structure or metabolism These fundamental problems are considered in more detail later when each type of disease is dealt with separately

From the point of view of diagnosis the presence in a patient suffering from hæmolytic anæmia of an intrinsic erythrocyte defect is indicated by the unimpaired survival in the patient of transfused normal corpuscles and less specifically by an increased tendency of the erythrocytes of the patient to undergo lysis or behave abnormally *in vitro* under certain specified conditions (see Chapter I pp 22–29)

HEREDITARY SPHEROCYTOSIS

Synonyms La microcythémie (Vanlair and Masius 1871) hereditare chronischen Ikterus (Minkowski 1900) L ictère congenital de l adulte (Chauffard 1907) hamolytischen Ikterus (Gansslen 1922) konstitutionelle hamolytische Anamie (Sphero cytenanamie Kugelzellen anamie) (Naegeli 1931) acholuric jaundice (Campbell 1925–26) spherocytic icterus (Krumbhaar 1936) familial hæmolytic anæmia (Dacie 1943) hereditary spherocytosis (Committee for Clarification of Nomenclature 1950)

Of the synonyms referred to above *hereditary spherocytosis* seems the most appropriate The title refers to the inherited nature of the disease as well as to a fundamental laboratory sign—spherocytosis

History The first significant contribution to the literature on hereditary spherocytosis appears to be that of Vanlair and Masius who in 1871 gave a remarkably accurate description of the disease under the title la microcythémie They recognized that some of the erythrocytes of their patient were small and spherical in character and a little more deeply coloured and suggested that these were corpuscles on their way to destruction (globules atrophiques) and that excess bile pigment was derived from them The illustration from their paper is reproduced as Fig 24 Vanlair and Masius s work has not received the recognition that it deserves

No other notable contribution was made for almost 20 years Then in 1890 Wilson gave an account to the Clinical Society of London of six members of a family in whom a condition in which an enlarged spleen accompanied by a sallow or subicteric com plexion appears as an hereditary condition Next a further

CHAPTER 2

THE CONGENITAL HÆMOLYTIC ANÆMIAS

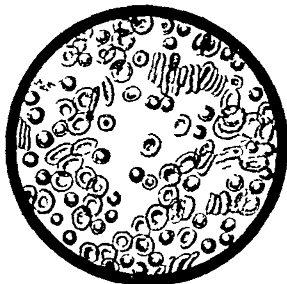
I HEREDITARY SPHEROCYTOSIS

A FAMILIAL form of jaundice was recognized by physicians towards the end of the nineteenth century (Murchison, 1885 Wilson 1890 Wilson and Stanley 1893) Wilson and Stanley s account was particularly important and undoubtedly referred to hereditary spherocytosis the splenomegaly the jaundice of the skin and conjunctivae the periodic attacks of deeper jaundice associated with biliary colic the inherited nature of the disease its chronicity and the fact that it was compatible with a long life—all these features were well described It was left to Le Gendre (1897) and Hayem (1898) to show that this type of jaundice was *acholuric* with bile in the plasma but not in the urine In 1900 followed Minkowski s well known description of eight cases of jaundice in three generations in this paper most of the salient clinical features of hereditary spherocytosis were well described

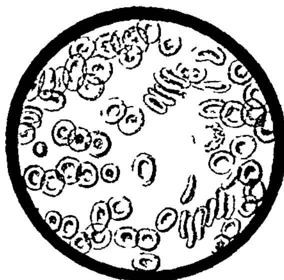
It is now realized that the disorder (hereditary spherocytosis) so clearly described by Wilson and Stanley (1893) and by Minkowski (1900) is but one of a series of distinct types of con genital hæmolytic disease In this and the following four chapters descriptions will be given of five major types *hereditary sphero cytosis hereditary elliptocytic hæmolytic anæmia congenital non spherocytic hæmolytic anæmia Mediterranean anæmia* and *sickle cell anæmia* Other less clearly defined types exist and these will also be briefly considered

All types of congenital hæmolytic anæmia depend upon an inherited abnormality of the patients erythrocytes the different diseases being distinguished by differences in the nature of the erythrocyte abnormalities and in the modes of inheritance In each type the result of the abnormalities is that the life span of the patients corpuscles is shortened All grades of cellular defect are found and this leads to marked differences in the severity of the anæmia from which the patients suffer

The nature of the erythrocyte defects is in most instances poorly understood It is known however that in sickle cell anæmia the molecules of hæmoglobin are abnormal and that in Mediterranean anæmia there is a defect in hæmoglobin synthesis



I



II

FIG 24 Reproduction of Vanlair and Masius's illustration to their paper De la microcythémie (Bull Acad méd Bel 5 3rd series 15 1871)

I is a drawing of the patient's blood

II is a drawing of control normal blood

and more detailed account was given by Wilson and Stanley (1893) and anæmia was recognized as an important feature of the disease Significantly they concluded that no doubt can be entertained that the splenic disease is accountable for this One of the patients died her spleen was examined and found to be firm and dark red on section and microscopic sections showed it to be engorged with blood cells Death was considered to have been due to active hæmolysis of splenic origin

A detailed account of Wilson s papers is given by Campbell (1925-26) who examined the only survivor of Wilson s six patients and confirmed the diagnosis of hereditary spherocytosis

Wilson s descriptions were followed in 1900 by the better known report of Minkowski Minkowski s paper was soon followed by those of Gilbert Castaigne and Lereboullet (1900) in France and by that of Barlow and Shaw (1902) in England Barlow and Shaw recognized that their cases were probably similar to those of Wilson Their report is interesting in that they record the presence of ulcers on the lower part of the leg in both their patients In America hereditary spherocytosis was first described in detail by Tileston and Griffin (1910) and by Thayer and Morris (1911)

In the last forty years the disease has been reported from many parts of the world and it now has a voluminous literature Reviews and monographs include those of Tileston (1922) Gansslen Zipperlen and Schuz (1925) Campbell (1925-26) Meulengracht (1922 1938) Bamatter (1932) Cheney and Cheney (1934) Vaughan (1936) Gripwall (1938) Dacie (1943) and Young Izzo and Platzer (1951)

CLINICAL FEATURES

Inheritance According to Meulengracht (1921) Plate (1913) was the first to suggest that hereditary spherocytosis was inherited as a Mendelian dominant Meulengracht himself investigated seven families in Stockholm He found that the healthy members of the families did not transmit the disease and with one exception that the disorder was always inherited through an affected parent Meulengracht attributed the exception to mutation Subsequent workers have confirmed the general truth of Meulengracht s observations (*Campbell and Warner 1925-26 Race 1942* Young Izzo and Platzer 1951 Abrams and Battle 1952)

The most recent detailed study is that of Race (1942) who examined 183 members of 26 different families in which the disease had occurred He confirmed that the inheritance followed the Mendelian dominant

pattern although there was a deficiency in the expected number of affected siblings Race attributed this to two factors an unusually high miscarriage rate and infant mortality of affected compared with unaffected siblings and variation in penetrance leading to mild and easily missed forms of the disease In 4 out of the 20 families studied by Race both parents of an affected propositus were apparently unaffected in 3 of the families all of 16 other relatives studied were also unaffected Race considered that mutation was a possible but unlikely explanation and that the observation gave some support for an acquired form of the disease Two of Race's patients (with both parents unaffected) were studied by the author in 1938 In these at least there seemed good evidence that the disease was congenital—both propositi were small children and the disease in each was typical in every way If mutation is in fact improbable it seems that limitation of penetrance in a carrier parent is the more likely explanation It is well known for instance that the disease in an active form may be transmitted by a parent who shows only minimal signs of the disease In the studies of both Race (1942) and Young *et al* (1951) there was no deficiency in the expected numbers of affected children of propositi although the number of affected siblings was below expectations

In one of Race's families (No 26) two probable heterozygotes who were first cousins married This would be expected to lead to the homozygous state in one in four of their children Two miscarriages occurred and Race suggested that these may have been the homozygotes and that the gene was lethal when homozygous However Bernard Boiron and Stager (195[illegible]) have recently reported studies on a family of 13 children all of whom suffered from varying degrees of anaemia jaundice and splenomegaly The father was also affected but the mother and her relatives were apparently normal Bernard and his colleagues considered that the most likely explanation for all 13 children being affected was that their father was homozygous for hereditary spherocytosis Unfortunately this could not be proved as clear evidence of jaundice was only found on the maternal side of his family

Race and Incidence Hereditary spherocytosis is an abnormality probably not confined to any particular race However it is certainly best known as a disease affecting people of European origin It is rare in negroes but probably not as rare as was at one time thought All the cases so far recorded in negroes seem to have been discovered in America (Scherer and Cecil 1945 Goodman and Cates 1947 McCormack and Simon 1948 Butterworth Kracke and Riser 1950) The disease has been reported by Salah (1936) in Egyptians and by Stransky and Davis Lawas (195[illegible]) in the Philippines Its true incidence in races other than European is not known In Britain it cannot be considered a rare disease The low mortality and the excellent results of splenectomy (*q v*) suggest that with a steady mutation rate the incidence of the disorder is likely to increase



Fig 25 Several bilateral chronic ulceration of the legs in a patient with hereditary spherocytosis Female aged 59 (Reproduced by courtesy of Dr A Gilpin)



Fig 26 Severe chronic ulceration of the left leg of a patient with hereditary spherocytosis Male aged 48 (Reproduced by courtesy of Dr A Gilpin)

genetic linkage with sex blood groups ability to taste phenyl thiocarbamide ability to secrete the ABO antigens in saliva eye colour or ear lobe attachment (Race 1942)

There are a few reports of the occurrence of endocrine abnormalities in association with hereditary spherocytosis Freymann (1922) described two brothers and a sister with tower skull delicately formed bones and hypogenitalism and Curschmann (1923) two patients with signs of hypogenitalism and infantilism More recently Falconer (1936) referred to a girl with signs of ovarian hypofunction and obesity and Debré and his colleagues (1938) to an occasional tendency to backwardness in physical and sexual development As mentioned on p 51 Bernard and co workers (1952) described a family of 13 affected children in nine out of the 13 children there was clear evidence of physical and mental retardation in four of them this amounted to true infantilism They all improved remarkably after splenectomy

Symptoms and Physical Signs

Patients with hereditary spherocytosis usually present with jaundice signs of anæmia and an enlarged spleen They may give in addition a history suggestive of gallstones Occasionally they complain of intractable leg ulcers As mentioned above some patients make no complaint the disease being discovered accidentally in others one or more of the main clinical features may be absent

Jaundice The jaundice is characteristically *acholuric* the direct van den Bergh reaction is almost always negative and the urine contains urobilin but not bile The degree of bilirubinæmia varies usually the plasma level lies between 1 and 4 mg per 100 ml Occasionally despite other signs of active hæmolysis the plasma bilirubin level is within the normal range i e 0 8 mg per 100 ml or less (King 1951) in such cases the liver is presumably particularly efficient in excreting the excess bilirubin formed Jaundice is rarely noticeable in children in the first few years of life (Debré *et al* 1938) Usually it is not until an affected child has reached school age or adolescence that jaundice appears—the early onset in the large family of Bernard Boiron and Estager (1952) is exceptional

Plasma bilirubin levels greater than 4 mg per 100 ml are infrequent in some patients high bilirubin levels are due to hæmolysis occurring at a particularly rapid rate in others they appear to be the result of the liver being relatively inefficient in excreting bilirubin Bile is occasionally found in the urine This

Age and Sex The disease is not sex linked Being congenital its presence is most frequently diagnosed for the first time when the patient is a child or young adult However there are numerous exceptions to this and it is by no means uncommon for the disorder to be unrecognized in early childhood Debré Lamy Sée and Schrameck (1938) reporting on 20 cases found that, whereas the disease was diagnosed in each case before the age of 14, in only three patients had the diagnosis been made before the age of four years Exceptionally the diagnosis may be made for the first time in an elderly subject attending hospital for some unrelated complaint or because one or more of his children have been found to be affected An extreme example of delayed diagnosis is illustrated by one of the family pedigrees published by Race (1942 family 9) The propositus of this family was a man aged 77 who was found to have a palpable spleen and slight anæmia when attending hospital for bronchitis Subsequently two of his children and a grandson were also found to be affected they considered themselves to be healthy although in fact they had the disease in a more severe form than had the propositus

On the other hand hereditary spherocytosis has been diagnosed in infants soon after birth (Hawksley 1936 Conrad and Schmidt 1946 Macaulay 1951 Bernard *et al* 1952) Usually this has been done when one of the parents or a previous sibling has been known to be affected and the disease has been particularly looked for In general the more severe the disease the more likely is it that the diagnosis will be made at an early age

Associated Abnormalities Gansslen Zipperlen and Schuz (1925) stressed the association between hereditary spherocytosis and other congenital abnormalities Gansslen thus referred to the hamolytische Konstitution ' He particularly stressed the occurrence of tower skull brachycephaly eye abnormalities polydactyly brachydactyly and infantilism Hansen and Klein (1934) added other abnormalities which they thought characteristic such as arched palate broad base of nose squint and dental abnormalities Other authors such as Meulengracht (1938) Gripwall (1938) Debré and co workers (1938) have not seen associated abnormalities with anything like the frequency that Gansslen and Hansen and Klein reported them Of Race s patients only three had gross associated abnormalities congenital absence of a hand in one patient cervical ribs in another and mental deficiency in another It seems likely therefore that in most families the incidence of skeletal abnormalities is not higher than in the general population Nor is there any evidence for

cytosis develop gallstones (Cheney and Cheney 1934 Bates and Brown 1952) They may be found even in children (Gairdner 1939) The stones are a potent source of trouble in later life and not infrequently lead directly or indirectly to the death of the patient Sometimes the presence of the hæmolytic anæmia itself is first discovered in a surgical ward the patient having been admitted for surgical treatment of a complication of gallstones The gallstones are of the pigment variety and presumably develop as the result of the increased concentration of bilirubin in the bile If their presence leads to cholecystitis or cholangitis stones of mixed type containing bile pigment and cholesterol may form Mixed stones are radio opaque pure pigment stones are not

Leg Ulcers Intractable ulcers of the leg not associated with varicose veins are a remarkable and quite frequent complication of hereditary spherocytosis (Figs 25 and 26) They were recorded as early as 1902 by Barlow and Shaw in a mother and her son As a rule the ulcers heal quickly after splenectomy (Vaughan 1936 Leger and Orr 1940) They are usually found in the old or middle aged patient Dedichen (1931–32) however reported crural ulcers in three young men (two of them brothers) aged 15 17 and 17 years respectively In each case the ulcers healed after splenectomy Another example in a woman aged 20 was described by Taylor (1939) *Here too the ulcer healed after* splenectomy

The ulcers are generally bilateral and nearly always start well above the medial malleolus In severe cases they may extend almost completely round the leg and also upwards for a consider able distance (Figs 25 and 26) They are quite indolent and are associated with pigmentation of the surrounding skin The presence of the ulcers is not diagnostic of hereditary spherocytosis for they are not uncommon in sickle cell anæmia and may rarely be met with in other chronic diseases associated with splenomegaly (Gendel 1948)

Blood Picture

Erythrocytes As already referred to (p 10) the erythrocytes in hereditary spherocytosis tend to be more spheroidal and less disc like than normal corpuscles The mean diameter of the cells is less than normal and their breadth (thickness) greater than normal the normal biconcavities are less marked It must be emphasized that the extent of the cellular abnormality varies from case to case and that there is in addition a considerable variation in the degree of spherocytosis in the cell population of

is usually due to biliary obstruction resulting from pigment gallstones Less commonly it is found in severe hæmolytic crises in the course of which liver damage sometimes develops In the series of patients described by Young Izzo and Platzer (1951) the pre operative bilirubin levels ranged from 0 6 to 5 7 mg per 100 ml the mean bilirubin level was 2 0 mg per 100 ml (see also Table 3)

Anæmia Anæmia in hereditary spherocytosis is very variable in degree it is unusual for it to be extremely severe not uncommonly it is slight or even absent In most patients the hæmoglobin level lies between 7 5 g and 14 g per 100 ml As a rule the rates of hæmolysis and regeneration are sufficiently stable for each patient to maintain a fairly steady hæmoglobin level for long periods

Minor Hæmolytic Crises It is characteristic of the disease that from time to time the jaundice deepens and the anæmia increases the patient may then complain of abdominal pain and develop pyrexia Vomiting is not uncommon and the spleen may increase in size In children unexplained pyrexia and tachycardia with abdominal pain and vomiting occurring at intervals and not associated with obvious jaundice may be the presenting symptoms of the disease (Debré *et al* 1938) After a few days or a week or so the exacerbation usually passes off and the patient s hæmo globin will then rise to about its usual level and the jaundice will diminish Minor crises such as these often follow intercurrent infections on other occasions there appears to be no obvious cause More serious crises occasionally develop these crises which generally seem to be due to a sudden failure in erythro poiesis are of special interest and will be dealt with in a later section (p 68)

Splenomegaly The spleen is probably invariably enlarged and it is uncommon to find it impalpable—it was so in six out of the 29 patients of Young and co workers (1951) In affected children a palpable spleen seems to be a particularly constant sign (Debré *et al* 1938) it may in fact be the only certain physical sign of the abnormality in a child or sibling of a known patient Usually the lower edge of the spleen is palpable somewhere between the left costal margin and the level of the umbilicus In consis tency the spleen feels moderately firm occasionally it is tender on palpation The spleen generally moves freely on respiration and at operation it is usually free and not adherent The histology of the spleen is considered on p 70

Gallstones Many patients suffering from hereditary sphero

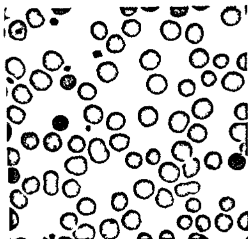


Fig 28 Photom crograph of a blood film of a patient suff ring from hereditary sph rocyto is I mal a 11 The round contours and deeper st n g of the m o t sph erocytic cells i well shown × 00

any particular patient It seems probable that as the erythrocytes mature they become more and more spherocytic and that the youngest cells (the reticulocytes) are the most disc like (lepto cytic) Nevertheless it has been shown that the reticulocytes in hereditary spherocytosis although thin discs, are less disc like and have smaller diameters than normal reticulocytes (Paulino

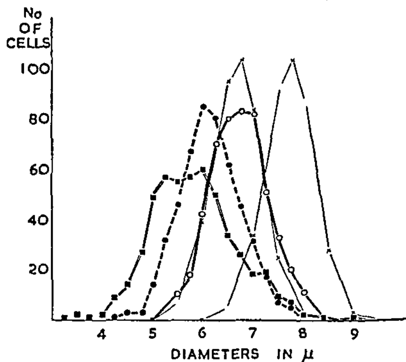


FIG 27 Erythrocyte-diameter distribution curves (Price Jones curves) made from the dried films of the peripheral blood of three patients with hereditary spherocytosis o—o is a curve of a mild example of the disease • • and ▪==▪ are curves of typical cases of hereditary spherocytosis The thin continuous lines indicate the maximum and minimum normal curves (Reproduced from Practical Hæmatology London Churchill 1950)

1949) The contrast in size between the spherocytes and the less densely staining more flattened cells some of which are reticulo cytes is shown in the stained blood film illustrated in Fig 28

The dimensions of the spherocytes of hereditary spherocytosis have been repeatedly studied Figures for mean cell diameter (M C D) mean cell thickness (M C T) and mean cell thickness diameter ratio are to be found in papers by Vaughan and Goddard

TABLE 3 *Hæmatological data and the effect of splenectomy on patients suffering from hereditary spherocytosis*
(The figures in parentheses indicate the number of patients investigated)

Pre- or Post splenectomy	Erythrocytes (millions per c mm.)		Haemoglobin (g per 100 ml)		M C V μ³		M C H C %		Reticulocytes (maximum count %)		Serum Bilirubin, mg. per 100 ml	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Pre	2 1–4 9 (39)	3 84	7 3 15 7 (18)	10 9	0–99 (33)	83 5	34–40 (18)	37 2 **	3–79 (34)	14 6	0 8–5 0 (10)	2 1
Post	3 9–6 0 (10)	4 92	1 9–17 0 (14)	14 7	67–98 (21)	84 8 *	3 –38 (14)	35 3	0 5–3 1 * (20)	1 4	0 3 1 6 (10)	0 74

Three of the 20 patients had counts exceeding 2
The difference between these two means is highly significant ($t = 3.8$, $P < 0.01$)
The difference between these two means is not significant ($t = 0.7$, $P > 0.05$)

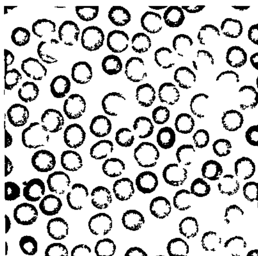


Fig 29 Photomicrograph of a blood film of Case 8 of Dacie *et al* (1953) showing typical slight spherocytosis (see text p 67) × 700

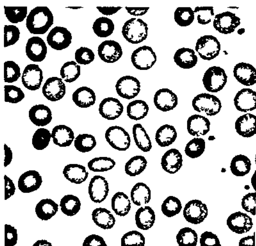


Fig 30 Photomicrograph of a blood film of Case 7 of Dacie *et al* (1953) showing slightly oval cells (see text p 67) × 700

count is markedly raised particularly if the patient is seriously anæmic Other things being equal normoblasts are found in greater numbers in the blood of children than in the blood of adults

Osmotic Fragility

Ever since the pioneer observations of Chauffard (1907) great interest has been taken in the phenomenon of the increased

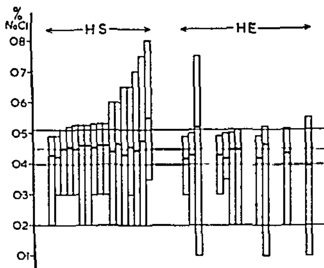


Fig 31 The results of osmotic fragility tests in 17 cases of hereditary spherocytosis (before splenectomy) (H S) and in 11 cases of hereditary elliptocytosis (H E)
The horizontal lines show the limits of the normal range and the double line the normal range of the M C F The observations on each patient are represented by an upright rectangle the short horizontal black bars show the M C F

osmotic fragility of the erythrocytes of hereditary spherocytosis Indeed at one time an increase in fragility almost came to be considered diagnostic of hereditary spherocytosis As already explained it is now recognized that this view was erroneous for the increased fragility depends upon spherocytosis which can be due to several causes

As discussed on p 23 the results of fragility tests have been generally reported either by recording the concentrations of saline which (a) cause just detectable lysis and (b) complete lysis or

(1934) Hawksley and Bailey (1934) Heilmeyer (1936) Hawksley (1936) Vaughan (1937) Gripwall (1938) Mogensen (1938) and Young Izzo and Platzer (1951) In 5 personally studied cases the average mean cell diameter (measured on dry films) was 6 4 μ (normal mean 7 2 μ) and the mean cell thickness (calculated from the M C D and M C V) was 2 6 μ (normal mean 2 μ) the average thickness diameter ratio was 0 40 (normal mean 0 28) The microcytosis (and also the increased anisocytosis) can be well demonstrated if Price Jones curves are drawn (Fig 27) The mean corpuscular volume is usually within the normal range before splenectomy the mean value in 33 of the writer s cases was 83 5 c μ and the range 70 to 99 c μ after splenectomy in 21 cases the mean was 84 8 c μ and the range 62 to 98 c μ (Table 3)

Spherocytosis can be appreciated if wet preparations of blood are examined (Fig 24) Gripwall (1938) and Dameshek (1939) have drawn attention to the unusual irregularity of the rouleaux which form in blood containing spherocytes the abnormal cells not fitting together as regularly and as tightly as do normal corpuscles

The hæmoglobin content of spherocytes is normal the hæmo globin concentration is slightly above the normal range (Table 3) (see later under *Chemistry of Spherocytes*) In stained films spherocytes appear as small relatively deeply staining cells As a rule there is no trace of central pallor (Fig 28) Their diameters vary considerably the contours are usually conspicuously rounded Their size and staining are best appreciated when they are compared in the same field of a blood film with the larger less spherocytic reticulocytes which stain diffusely basophilic with the Romanowsky dye

Reticulocytes In hereditary spherocytosis reticulocytes are normally present in far larger numbers than in health the count usually being between 5% and 20% The reticulocyte counts of the author s cases (before splenectomy) are shown in Table 3 Usually the reticulocyte counts remain at high levels throughout the patients lives unless splenectomy is carried out Occa sionally however for reasons which are at present obscure erythropoiesis in the marrow may become greatly reduced or even completely suspended The peripheral reticulocyte count then falls to low levels and the patients may quickly go into an aplastic (or anæmic) crisis (see p 68)

Erythroblastæmia Normoblasts are not commonly present in the peripheral blood of patients with hereditary spherocytosis Small numbers however may be found when the reticulocyte

count is markedly raised particularly if the patient is seriously anæmic Other things being equal normoblasts are found in greater numbers in the blood of children than in the blood of adults

Osmotic Fragility

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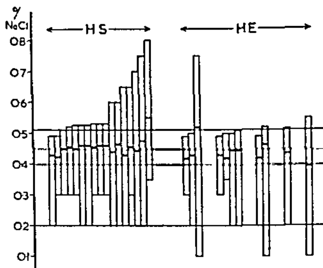


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The horizontal lines show the limits of the normal range and the double line the normal range of the M C F The observations on each patient are represented by an upright rectangle the short horizontal black bars show the M C F

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As discussed on p 23 the results of fragility tests have been generally reported either by recording the concentrations of saline which (*a*) cause just detectable lysis and (*b*) complete lysis or

more completely by recording the percentage of hæmolysis caused by each saline concentration used The results of a quantitative test form a curve when plotted on graph paper It is conventional to plot the percentage lysis as ordinate against the corresponding concentration of hypotonic saline as abscissa (Fig 17 p 24) All grades of increase in osmotic fragility are found in hereditary spherocytosis and in a small proportion of cases the

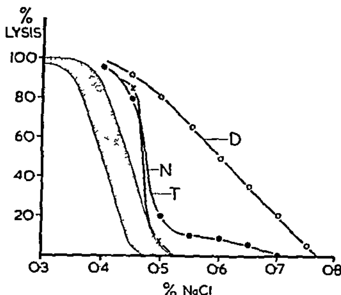


FIG 3. Types of osmotic fragility curves in hereditary spherocytosis (redrawn for Dacie 1943)
N Normal type curve
T Tailed curve
D Diagonal curve
The shaded area represents the normal range

result falls just within the upper limit of the normal range The results of tests carried out on patients recently studied by the author are shown in Fig 81

Dacie (1943) reporting on the curves obtained with the blood from 24 patients suffering from hereditary spherocytosis [1] noted certain differences in the form of the curve in different patients The commonest type of curve was a tailed one with curves of this sort hæmolysis was first detected with saline of concentrations between 0 76% and 0 58% and only gradually increased in amount with diminishing saline concentration until a point was reached at which 10 to 20% of the erythro-

[1] It is probable in retrospect that one and possibly two of these patients were suffering from acquired hæmolytic anæmia

cytes were lysed Beyond this point the curve became abruptly steeper and of approximately the same shape as the curve of a normal person The cell populations producing curves of this sort are clearly heterogeneous and include small proportions of unusually fragile cells In 6 patients diagonal curves were recorded here hæmolysis was first perceptible with saline concentrations varying between 0 80% and 0 68% and increased fairly steadily as the concentration of saline was reduced In 5 patients in whom the increase in fragility was slight the shape of the curve was normal or almost normal and there were only very small tails of fragile cells Further experience has confirmed

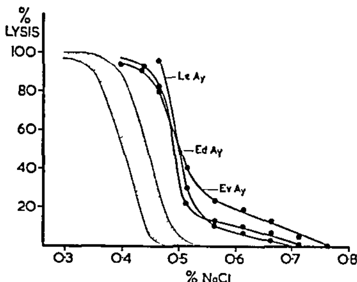


Fig 33 Osmotic fragility curves of three patients with hereditary spherocytosis all belonging to the same family The shaded area represents the normal range

the general truth of these observations but it is clear that curves intermediate in type between the extremes illustrated in Fig 32 occur It seems likely that a patient retains his own characteristic type of curve for long periods and that members of the same family more often than not have the same type of curve (Figs 33 and 34) There is however no close correlation between either the initial or the median fragilities and the patients erythrocyte counts although it is true that in general those patients with the greatest increases in osmotic fragility are the most severely affected clinically

Osmotic Fragility after Incubation for 24 Hours at 37 C (Incubation Fragility) Emerson Shen and Castle (1946) Emerson Shen Ham and Castle (1947) Young (1947) Maier

(1950) and Varadi (1951) observed that the increase in osmotic fragility resulting from incubation at 37° C was more marked in hereditary spherocytosis than in normal subjects. Later Young, Izzo and Platzer (1951) published details of their observations on 17 patients before splenectomy and concluded that the incubation test was particularly useful in detecting the slightest grades of abnormality. In several instances the fragility of the blood suspected to be abnormal was significantly greater after incubation than that of normal controls whereas there had been no significant difference before incubation. Curves illustrating the changes in a typical case are shown in Fig. 19 (p. 25). While there is no doubt as to the general usefulness of the 'incubation fragility' test it seems doubtful whether it will always permit the differentiation of the mildest examples of hereditary spherocytosis from normal. Dacie, Mollison, Richardson, Selwyn and Shapiro (1953) recorded the history of a man aged 31, a member of a family known to have hereditary spherocytosis of a slightly atypical type (see p. 67) who was neither anæmic nor jaundiced but whose spleen was palpable. On incubation the fragility of his erythrocytes still remained at about the upper range of the normal.

The unusual increase in osmotic fragility which results from the incubation of the blood of patients with hereditary sphero-cytosis is associated with an increased rate of spontaneous hæmolysis (Selwyn and Dacie, 1954) (see below).

Spontaneous Lysis on Incubation at 37° C (Autohæmolysis)

Ham and Castle (1940a and b) reported that when blood from a patient with hæmolytic jaundice was incubated at 37° C hæmolysis occurred after 12 hours although blood from a normal subject did not undergo hæmolysis for 32 hours. Dacie (1941) reported observations on 10 patients. Amounts of lysis varying from traces to 5% lysis were observed after 24 hours' incubation and up to 50% lysis in 48 hours. With normal blood only traces of lysis were to be seen after 24 hours and not more than 5% after 48 hours' incubation. It was also shown that in hereditary spherocytosis spontaneous lysis was a property of defective erythrocytes, for lysis took place at an accelerated rate in saline suspensions of cells in the absence of plasma and as rapidly in normal plasma as in autogenous plasma; lysis was somewhat slowed in plasma or serum which had been previously heated at 56° C for 30 minutes. A general correlation was noted between rates of lysis and the degree of increase in osmotic fragility (before incubation). This work has since been confirmed by Caroli, Etévé, Paraf and

Robineau (1949) Young Izzo and Platzer (1951) and Young and Miller (1953) Selwyn and Dacie s (1954) investigation was undertaken to try to find out what was the underlying cause of the rapid lysis of spherocytes on incubation *in vitro* Several interesting facts were established The spherocytes of hereditary spherocytosis swell and take in sodium at the same rate as normal corpuscles they lose potassium at a normal or slightly accelerated rate These changes are markedly slowed in the presence of glucose as are the changes in normal corpuscles Lysis is not correlated with swelling of the corpuscles as suggested by Ham and Castle (1940a and b) but rather with the rapidity of increase in fragility on incubation Selwyn and Dacie (1954) concluded tentatively that lysis depended upon a defective cell membrane which underwent a degenerative irreversible shrinkage more rapidly than normal The nature of the membrane abnormality and the processes involved in its degeneration have yet to be established (see also p 82)

Mechanical Fragility

Spherocytes were reported by Shen Castle and Fleming (1944) Maier (1950) and Matthes (1950) to be unusually easily lysed by mechanical trauma The most extensive data on this point is to be found in Young Izzo and Platzer s (1951) paper Young and his colleagues studied 18 patients (before splenectomy) and showed that the mechanical fragility of their corpuscles was on the average 4 to 5 times that of normal controls after incubation the average mechanical fragility of the patients corpuscles was about 3 times that of the controls

Serology

Boorman Dodd and Loutit (1946) and Loutit and Mollison (1946) reported that the direct antiglobulin (Coombs) test was negative in patients with hereditary spherocytosis The observations of most later workers including those of the author have confirmed this Young Izzo and Platzer (1951) for example reported negative results in 28 patients Positive reactions have however been reported by Singer and Motulsky (1949) Wright Dodd and Bouroncle (1949) and Wright Dodd Bouroncle Doan and Zollinger (1951) It is possible that some of these positive reactions have been due to the use of an unsuitable technique However in a few patients in hæmolytic crises it seems probable that antibody development leading to autosensitization may have been superimposed upon the original congenital disease

(1950) and Varadi (1951) observed that the increase in osmotic fragility resulting from incubation at 37 C was more marked in hereditary spherocytosis than in normal subjects Later Young Izzo and Platzer (1951) published details of their observations on 17 patients before splenectomy and concluded that the incubation test was particularly useful in detecting the slightest grades of abnormality In several instances the fragility of the blood suspected to be abnormal was significantly greater after incubation than that of normal controls whereas there had been no significant difference before incubation Curves illustrating the changes in a *typical case are shown in Fig 19 (p 25) While there is no doubt* as to the general usefulness of the incubation fragility ' test it seems doubtful whether it will always permit the differentiation of the mildest examples of hereditary spherocytosis from normal Dacie Mollison Richardson Selwyn and Shapiro (1953) recorded the history of a man aged 31 a member of a family known to have hereditary spherocytosis of a slightly atypical type (see p 67) who was neither anæmic nor jaundiced but whose spleen was palpable On incubation the fragility of his erythrocytes still remained at about the upper range of the normal

The unusual increase in osmotic fragility which results from the incubation of the blood of patients with hereditary sphero cytosis is associated with an increased rate of spontaneous *hæmolysis (Selwyn and Dacie 1954) (see below)*

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Bile pigment Metabolism in Hereditary Spherocytosis

The usual range of plasma bilirubin levels in hereditary spherocytosis has been referred to on p 53 Urobilinogen excretion in the fæces is characteristically increased and may be many times the normal (Goldschmidt Lepper and Pearce 1915 Watson 1937 Barker 1938 Crosby and Akeroyd 1952)

Watson s (1937) data are the most extensive Ten of his patients suffered from hereditary spherocytosis their excretion of urobilinogen varied from 136 to 2 475 mg per day the average was approximately 900 mg (about 6 times normal) Barker (1938) studied 3 patients their daily excretion ranged from 500 to 1 087 mg Watson *made the point that the excretion of uro*bilinogen in the urine is hardly raised in uncomplicated cases in his patients the total daily excretion ranged from 1 to 10 mg compared with the normal excretion of 0 6 mg When urobilinuria of marked degree occurred this was in Watson s view nearly always due to complications such as infection severe anæmia infarction the effects of anæsthesia or hemolytic crisis —all of which affected the function of the liver In one jaundiced patient with marked urobilinuria however none of these factors was apparently operating and it appeared likely that liver function was concurrently disturbed at least in respect of its power of excreting bile pigment

VARIANTS OF HEREDITARY SPHEROCYTOSIS

Mild Forms of the Disease Gansslen Zipperlen and Schuz (1925) and Campbell and Warner (1925–26) were among the first writers to stress the existence of very mild forms of the disease As a result of studies involving about 120 patients Gansslen and co workers described three main types of the disease a *complete form* a *compensated form*—35% of the patients without anæmia 5% with polycythæmia 40% without jaundice 30% without splenomegaly 10% without increased osmotic fragility and a *mild form* which they referred to as the leichte hamolytische Konstitution In this last group were placed healthy people with perhaps slightly increased degrees of anisocytosis of the erythrocytes minor fragility changes slight and inconstant bilirubinæmia but with no anæmia or splenomegaly Out of 68 members of a family comprising 161 persons in three generations Gansslen and his colleagues found ten completely healthy subjects eleven with the complete disease thirty four with the compensated disease and thirteen with the mild carrier form

(Dameshek and Bloom Case 6 1948 Mendes de Leon 1952 Young and Miller 1953) Mendes de Leon (1952) in studies on the effect of incubation on normal corpuscles suspended in patients sera reported that in nine out of 12 patients (before splenectomy) the 'hæmolytic activity' of the serum was increased After splenectomy the hæmolytic activity was normal The significance of these observations needs elucidation

Erythrocyte Chemistry in Hereditary Spherocytosis

V The few chemical observations available point to differences between hereditary spherocytes and normal corpuscles

Maizels (1936) concluded that the potassium and water concentrations of the erythrocytes were low in hereditary spherocytosis the sodium concentrations were normal and the hæmoglobin concentrations increased He pointed out that the *hæmoglobin and water concentrations* would have an inverse relationship and that the total cation concentrations would be dependent on the water concentration He also pointed out that as a small proportion of the cell water is bound by the hæmoglobin the high hæmoglobin concentration in spherocytes would result in a higher proportion of the cell water being bound Maizels studied three patients before and after splenectomy The hæmoglobin concentration in the erythrocytes fell by about 10% after operation and their water content increased slightly the potassium concentrations and cell volumes rose slightly

Erickson Williams Hummel Lee and Macy (1937) studied the erythrocytes of three children with hereditary spherocytosis (two after splenectomy) in one patient before splenectomy they found the cell potassium concentration to be high in the other two children examined after splenectomy the concentrations were normal *These observations* are at variance with those of Maizels (see also below)

Selwyn and Dacie s (1954) work was based on 10 patients and has confirmed Maizels s observations They found the hæmoglobin concentration to be high mean 37 g range 34–40 g per 100 ml cells (normal range 32–36 g per 100 ml cells) the cell water to be low range 65–69% (normal range 69–72%) the cell sodium to be normal range 8–12 m Eq /litre cells (normal range 8–12 m Eq /litre cells) and the cell potassium to be low range 75–95 m Eq /litre cells (normal range 100–114 m Eq /litre cells) After splenectomy there was a shift towards the normal (The effect of incubation on the chemistry of spherocytes has already been referred to (p 63))

Erickson and co workers (1937) also studied the corpuscular lipids in certain cases of hæmolytic anæmia In four patients with hereditary spherocytosis the total lipid per cell averaged 3.0 × 10^{-11} mg and in three patients after splenectomy 330 × 10^{-11} mg per cell These figures *suggested a definite deficiency in the lipid content of hereditary spherocytes* Crosby (1952) quoting the work of Erickson and co workers correlated this lipid deficiency with the reduced surface area of the spherocytes He suggested that in the maturation of the hereditary spherocyte there was a disproportionately great loss of surface area and presumably of the materials that comprised the cellular surface

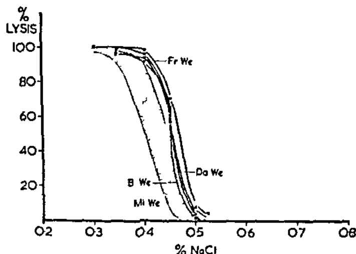


Fig 34 Osmotic fragility curves of four brothers all suffering from hereditary spherocytosis (Cases 1 to 4) The shaded area represents the normal range

Atypical Hereditary Spherocytosis It is hard to define the limits of a congenital disease so variable in its severity as hereditary spherocytosis However it seems likely that real minor variants or mixed cases occur Dacie and his colleagues (1953 Case 7) published details of a patient thought to be of this type

Two brothers and their sister all suffered from a mild inherited hæmolytic anæmia The erythrocyte osmotic fragility was at the upper limits of the normal range in all three (Their father who was also affected was said to have had erythrocytes of normal osmotic fragility) The elder brother s erythrocytes were slightly spherocytic and he seemed to be suffering from mild well compensated hereditary sphero cytosis (Fig 29) The only really definite abnormality in the younger brother was a palpable spleen Their sister however was anæmic with 11 3 g hæmoglobin per 100 ml 3 300 000 erythrocytes per c mm and 16 % reticulocytes Hæmolysis seemed to be moderately active *in vivo* The osmotic fragility of her erythrocytes however was found to be normal an unexpected finding Moreover many of her corpuscles were slightly but definitely oval in contour instead of being the rounded spherocytes that had been expected (Fig 30) It is possible that this patient had inherited the trait for hereditary elliptocytosis from her mother unfortunately it was not possible to confirm or refute this hypothesis The patient underwent splenectomy Sixteen months

Subsequent writers have tended to overlook the possibility of the extremely mild forms described by Gansslen although compensated cases have been well recognized On the other hand the diagnosis of leichte hamolytische Konstitution should not be made unless repeated careful studies have been carried out particularly as slightly increased anisocytosis is of frequent occurrence and small fragility changes are difficult to be certain about

There is some evidence which suggests that mildness of the disease may be a family characteristic For instance families have been observed in which anæmia has been slight and the *increases in osmotic fragility have been similar and minimal in* all of the affected members the fragility curves being normal or almost normal in shape (Wiedemann 1942 Discombe 1948 Young Izzo and Platzer 1951) It is not clear whether these cases represent a slightly atypical type of hereditary spherocytosis or whether the mildness of the disease is due to the influence of the unaffected parent on the expressivity of the gene

The author has observed a family of this type in which four boys *were affected with a mild form of the disease*

Case Reports Mild Hereditary Spherocytosis in Four Brothers (Cases 1 to 4)

The patients were boys aged 10 8 7 and 5 years respectively They had all been noticed to be intermittently mildly jaundiced although their general health had been good Their father and paternal grandmother had both undergone splenectomy for hæmolytic anæmia A paternal aunt aged 48 is also known to have been mildly anæmic and jaundiced The boys blood was first examined by Dr J C Selwyn in October 1950 None of the boys was significantly anæmic their osmotic fragilities were at the upper margin of the normal range the serum bilirubin levels ranged from 0 5 to [illegible] 2 mg per 100 ml and their reticulocyte counts from 0 5 to 6 6%

The boys were re examined in January 1952 All had coughs and *colds The eldest boy (Brian) was in bed he had felt sick for the last week* and had vomited occasionally The two elder boys Brian and Michael were found to have a reticulocytopenia (0 3% and 0 2% reticulocytes respectively) and to have falling erythrocyte counts they were in fact in a mild aplastic crisis (see p 68) The blood of the younger two boys (David and Francis) contained a raised number of reticulocytes and their condition caused no anxiety Brian and Michael were admitted into hospital A reticulocytosis developed in a few days and their anæmia thereupon improved spontaneously *The osmotic fragility of the blood of all four boys* was just outside the normal range the curves were of the normal upright type with only very small tails (Fig 34) Later all four boys underwent splenectomy with excellent results The histology of their spleens was typical of hereditary spherocytosis (see p 70)

of the bone-marrow particularly affecting erythropoiesis Owren concluded that crises in hereditary spherocytosis should be termed aplastic crises rather than hæmolytic crises

Dameshek and Bloom (1948) studied 7 patients 3 of them having been reported previously by Dameshek (1941) in these patients the crisis was interpreted as being due to the combination of a marked exacerbation of the hæmolytic mechanism with an arrest of maturation of the developing erythroblasts in the marrow The marrow inhibition was attributed to a pathologically hyperactive spleen Reticulocytopenia was marked in six of the patients all suffering from major crises and there were lesser degrees of granulocytopenia and thrombocytopenia In one patient (Case 7) reticulocytes were reported to be absent from the peripheral blood for at least [illegible] days Marked spherocytosis was a feature of the crises in all Dameshek and Bloom s patients Serial bone marrow studies were carried out on one patient (Case 7) From this it appeared that at the height of the reticulocytopenia in the peripheral blood there was a maturation arrest affecting erythropoiesis It is possible though that the appearances were those of early recovery of marrow function and that a puncture done earlier in the crisis would have shown marrow hypoplasia The exaggerated spherocytosis in the peripheral blood can be explained as the direct consequence of diminished formation as the circulating cell population would become increasingly older and more spherocytic as time passed

Gasser (1950 1951) described the occurrence in children of aplastische Erythroblastenkrisen (akute Erythroblastopenie) in the course of various illnesses including hereditary spherocytosis The sequence of events is well illustrated in his 1950 paper Like Owren he noted the disappearance of reticulocytes and erythroblasts in the bone marrow the marked reticulocytopenia in the peripheral blood and the rapid onset of anæmia He also recorded an increase in osmotic fragility and a fall in serum bilirubin as the anæmia progressed

The cause or causes of an aplastic crisis have not been established The familial incidence suggests strongly that infections may be precipitating factors Whether the depression of the bone marrow results from the direct action of virus or toxin or is brought about indirectly by some as yet unknown mechanism is uncertain It certainly seems that compensatory erythropoiesis in hereditary spherocytosis is delicately poised Gasser s observations suggest that a temporary depression of erythropoiesis is not uncommon in children as a result of infections and intoxications however it is only in hæmolytic anæmia that the results are serious

PATHOLOGY

Bone Marrow The bone marrow is characteristically hyperplastic fat cells disappear partially or completely from the marrow of the flat bones (Fig 11 p 17) and red marrow extends into areas in the long bones normally fatty In children hyper

later she reported that she had been very well since the operation her hæmoglobin was 13 6 g per 100 ml and the reticulocyte count was less than 2%

Aplastic (Anæmic) Crises in Hereditary Spherocytosis

The occurrence of crises in the course of hereditary sphero cytosis has been known for many years (Tileston 1922 Dawson 1931 Dameshek and Bloom 1948) They were described as crises de déglobulisation by French writers in the early years of this century More recently it has been recognized that crises may affect several members of the same family concurrently or successively and that in some instances at least an obvious increase in hæmolysis with deepening jaundice is not the cause of the crisis

Examples of epidemic familial crises were described by Murray Lyon (1935) Scott (1935) Dedichen (1937) Dameshek (1941) Lyngar (1942) Horne Lederer Kirkpatrick and Leys (1945) Li Yoth and Osgood (1950) Marson Meynell and Tabbush (1950) Battle (1952) Ingham (1952) and Margolis (1953) It is noteworthy that Murray Lyon Scott Dameshek and Lyngar all remarked on the low reticulocyte counts of their patients at the height of their crises when they were most anæmic Scott (1935) correctly concluded that erythropoiesis was failing to keep pace with destruction and Dameshek (1941) suggested that the crisis might develop not only because of increased hæmolysis but also because of inhibition of the bone marrow due to unusual splenic activity Dameshek also remarked on the leucopenia that was present at the time of the crises and suggested that this might be due to a splenic influence also Detailed studies on the genesis of the crises have since been published by Owren (1948) Dameshek and Bloom (1948) and Gasser (1950 1951)

Owren described the course of the crisis in 6 patients four of them were members of the same family all became ill within a few days The other two patients belonged to different families In each case the patient suddenly developed pyrexia two patients had rigors one of them experienced abdominal pain with vomiting The pyrexia lasted about 10 days the patients temperature returning to normal at about the same time as the reticulocytes reappeared in the peripheral blood The patients jaundice was observed to decrease as they became more anæmic whilst the size of their spleens remained unaltered In all his patients Owren found a severe reticulocytopenia at the height of their crises—the counts varied from 0 to 0 3% In addition there was granulocytopenia the total neutrophil counts ranging from 760 to 2 400 cells per c mm and thrombocytopenia with counts ranging from 30 000 to 160 000 per c mm Bone marrow studies showed that the peripheral pancytopenia was a reflection of an acute hypoplasia

empty sinuses seen in histological preparations of spleens removed at operation are presumably filled with blood in life The question is whether or to what degree the extreme congestion of the pulp is an artefact Knisely (1936) claimed as a result of direct inspection of the spleens of anæsthetized small animals that all the blood was contained in life in the sinuses and that its presence in the pulp in fixed sections was due to agonal changes Knisely s conclusions were disputed by MacKenzie Whipple and Wintersteiner (1940) using a similar technique However Cripwall (1938) in reporting a study on patients with hereditary spherocytosis claimed that sections of small pieces of two spleens removed at the time of operation and rapidly fixed showed the sinuses to be well filled with blood and not compressed by an overloaded pulp (see also p 83 under *Pathogenesis*)

Other Organs The changes in other organs as revealed by studies on fatal cases are less characteristic There is often a moderate amount of hæmosiderosis of the liver but myeloid metaplasia is seldom seen The frequency of pigment gallstones has already been mentioned There is a variable amount of iron in the kidney (Turnbull 1936) The microscopical appearance of sections of a crural ulcer were described by Turnbull (1936) as a chronic inflammatory process

DIFFERENTIAL DIAGNOSIS

The most important diagnostic features of hereditary spherocytosis are as follows it is a congenital and hereditary disease the fundamental abnormality resides in the erythrocytes spherocytosis and increased osmotic fragility are characteristic although not diagnostic normal erythrocytes survive well after transfusion the results of splenectomy are excellent (see later) No disease presents a picture quite like this and when all the signs are present the diagnosis is easy Difficulty arises in the mild or atypical case for instance when the disease appears not to be congenital when the family history is negative when the osmotic fragility is normal and when the morphology of the erythrocytes is unusual or when the patient is seen for the first time in an aplastic crisis (see p 68) Most of these points have already been dealt with The fact that the patient is an elderly subject should not be allowed to weigh too heavily against the diagnosis of hereditary spherocytosis examination of relatives may reveal the disease in an unmistakable form (p 52) Nor should the absence of a positive family history be deemed too important in an obviously congenital case if the rest of the picture is typical It may be that the disease is really present in a relative but in such a mild form that its certain recognition is impossible

plasia of the marrow sometimes leads to widening of the diploe of the skull and radiological appearances rather similar to those seen in severe Cooley's anæmia (Caffey 1937) The marrow hyperplasia is due to proliferation of the normoblasts in severely anæmic patients they may be the predominant marrow cell Morphologically erythropoiesis is essentially normoblastic in type the developing cells being normal in size Mitotic figures are increased in number Megaloblastic change is extremely rare but has been observed for example in an elderly patient suffering from a concurrent folic acid deficiency possibly of dietary origin (Matthews 1954) It seems quite clear that in the otherwise healthy subject erythropoiesis can be maintained at many times the normal rate for many years without any deficiency of essential hæmopoietic factors developing

Extramedullary erythropoiesis has been observed from time to time Paravertebral masses were described by Dawson (1931) Bamatter (1932) and Hartfall and Stewart (1933) masses in the costovertebral angles by Cleave (1936) and paravertebral subpleural nodules by Turnbull (1936)

Spleen The spleen is always enlarged but the enlargement is rarely extreme The largest spleens are found in the most severely affected patients After excision the spleen of an adult patient is usually found to weigh between 500 and 2 000 g Infarcts are not usually found and adhesions if present are rarely extensive The vessels at the splenic hilum are not conspicuously large On section the spleen is characteristically a dark plum colour firm to the touch and looks as if it were deeply congested with blood the vessels and fibrous trabeculæ and Malpighian bodies are not usually conspicuous Microscopically the appearances are characteristic (Eppinger 1920 Meulengracht 1922 Thompson 1932 Turnbull 1936 Klemperer 1938) The Malpighian bodies are usually normal in size but are widely separated by a pulp filled with blood Most of the blood corpuscles are packed in the pulp cords in contrast the sinuses are often empty and may be lined by conspicuous almost cuboidal endothelial cells There is usually no increase in collagen although there may be a slight increase in argyrophil fibres Macrophages containing *erythrocytes are not easily found but there is usually* a moderate amount of hæmosiderin present in phagocytic cells or as a diffuse impregnation Areas of myeloid metaplasia are not commonly observed

The exact location in life of the erythrocytes in the spleen of hereditary spherocytosis has been a subject of controversy The relatively

Indications for Splenectomy It is generally agreed that the spleen should be removed from any patient suffering from typical hereditary spherocytosis who is continuously anæmic or has a clinical degree of jaundice or who gives a history of an aplastic crisis The results of the operation are so good and the mortality nowadays so low that the operation should be carried out in all patients except in the completely compensated and symptom free cases The spleen has been removed successfully in infancy (Conrad and Schmidt 1946) this should certainly be done if anæmia is so severe that repeated transfusions are essential The operation should however be postponed to late childhood if possible After the age of ten the sooner the operation is carried out the better for the longer hæmolysis continues the greater is the risk of complications arising as the result of gallstones Whether the gallstones if present should be dealt with at the time of splenectomy is a surgical problem and must be left to the discretion of the surgeon The loss of the spleen does not seem to affect the general health of the patient in any way and although there are a few reports of tuberculosis becoming manifest soon after the operation (Beckman and Jaderholm 1931–32) it is doubtful whether the removal of the spleen *per se* had anything to do with the development of the tuberculosis

Failure of Splenectomy

Accounts have been published from time to time of patients reputed to suffer from hereditary spherocytosis in whom splenectomy has been a failure In retrospect it is often extremely difficult to know exactly from what the patients were suffering In most cases the diagnosis was probably congenital non spherocytic hæmolytic anæmia or acquired hæmolytic anæmia in only a very few instances was the patient probably suffering from hereditary spherocytosis

(*a*) *Patients with Congenital Non spherocytic Hæmolytic Anæmias* It is now known that there are other congenital and hereditary hæmolytic diseases which differ fundamentally from hereditary spherocytosis in pathogenesis but which have been confused with it in the past Probably the commonest type of atypical congenital hæmolytic anæmia for which splenectomy has been carried out is the non spherocytic type (see p 104) Dacie and colleagues (1953) described for instance 4 patients all of whom had had their spleens removed without benefit It is probable too that the patients who failed to respond to splenectomy described by Edwards (1951) and Lemaire Loeper and Moschoutis (1952) belonged to this group

(*b*) *Patients suffering from Acquired Hæmolytic Anæmia* As has

by present methods alternatively there is the less likely possibility of a new mutation

It is undoubtedly true that exceptionally the osmotic fragility may be almost if not quite normal The author has seen examples of this In these cases the diagnosis is admittedly difficult for there may well be confusion between two distinct diseases mild *hereditary spherocytosis* and *congenital non spherocytic hæmolytic* anæmia if too much importance is given to the results of the fragility test alone In these cases it is important to investigate as many relatives of the patient as possible Study of the effects of incubation at 37 C on osmotic fragility and autohæmolysis may also help greatly in differentiation (see p 26)

TREATMENT OF HEREDITARY SPHEROCYTOSIS

Splenectomy

The late results of splenectomy in hereditary spherocytosis are almost uniformly excellent Gansslen (1922) reported that nine out of 10 patients were clinically cured—the one failure was a patient who died of a postoperative portal vein thrombosis Thompson (1930) reported uniform and permanent relief in 18 patients and Cowan (1936) the same good results in 20 patients More recently Welch and Dameshek (1950) reported that complete remissions resulted in 38 patients Edwards (1951) obtained excellent results in 24 out of 25 patients—the one failure is discussed later and Young Izzo and Platzer (1951) reported complete remissions in 16 patients Twenty four patients studied by the author have undergone splenectomy all have done well

According to Dawson (1931) the first successful splenectomy in hereditary spherocytosis was carried out unwittingly by Spencer Wells in 1887 His patient was a woman aged 27 who had had attacks of jaundice since 9 years of age She had an abdominal tumour which was thought to be a fibroid this however turned out to be a very large spleen Dawson reported that the osmotic fragility of her erythrocytes was still increased when he examined her blood about 40 years later Clinically she was then in good health Her son underwent cholecystectomy and splenectomy at the age of 14 his erythrocytes also were reported by Dawson to be fragile Splenectomy does not seem to have been performed again with benefit to the patient until Micheli s (1911) success in an acquired case stimulated other operators to carry out splenectomy in hæmolytic anæmias In England at a discussion at the Royal Society of Medicine *early in 1913* several successful operations were referred to (Wynther 1912–13) By 1922 Tileston was able to write in the congenital type of hemolytic jaundice a permanent cure may be predicted as the result of splenectomy

erythrocytes in supravitally stained preparations of the spleen and splenunculi—a highly unusual finding in hereditary spherocytosis

Dorn (1949) referred to a patient diagnosed as suffering from congenital hæmolytic jaundice who underwent splenectomy for a hæmolytic crisis A complete remission followed which lasted for 4 years Later hæmolytic anæmia reappeared Laparotomy revealed three small accessory spleens weighing in all not more than 5 g Sections showed many highly phagocytic clasmatocytes laden with erythrocytes The patient slowly improved following the removal of the spleens and it was not until 7 months later that the reticulocyte count fell to normal In this account there is no mention of a positive family history In retrospect it seems difficult to exclude the possibility that this patient s disorder was acquired and not congenital

More recently Loeb Seaman and Moore (1952) have published what appears to be a genuine instance of relapse after splenectomy in true hereditary spherocytosis In their patient a small piece of adherent spleen was known to have been left behind at the original splenectomy The patient did well for 7 years then he relapsed Radiography with the aid of thorotrast demonstrated a radio-opaque shadow 2 cm in diameter in the region of the spleen This was thought to represent hypertrophied splenic tissue derived from the fragment left behind at the original operation

Another possible example of relapse after splenectomy was reported by Freund (1932 Case 1) Freund s patient was a boy aged 10 years who was said to have been always jaundiced Blood tests showed microcytosis and a very great increase in osmotic fragility There was no obvious family history but the erythrocyte osmotic fragility of his mother was reported to be slightly increased Splenectomy was carried out after a temporary remission hæmolysis again became active At post mortem the most conspicuous finding was the great engorgement of the liver with blood

Blood Transfusion

It has quite often been stated that transfusion in hæmolytic jaundice is likely to result in severe reactions (e g Dawson 1931) It is not clear whether this applies to hereditary spherocytosis In the author s limited experience it has not proved to be so In some of the recorded examples of transfusion reactions it is likely that immune iso antibodies were present other patients may have been suffering from acquired hæmolytic anæmia As normal corpuscles survive well in the recipients after transfusion there seems no reason why transfusion reactions should occur with undue frequency It is possible however that serious reactions may develop in patients in severe crises if so it must be admitted that their cause is quite obscure Transfusion should however never be withheld from a seriously anæmic patient on the ground that a harmful reaction might occur The transfusion is much more likely to be life saving than otherwise (Dameshek 1941)

already been mentioned the blood picture in acquired hæmolytic anæmia of the auto antibody type may be very similar to that in hereditary spherocytosis In particular spherocytosis may be a well marked feature of the acquired disease It is difficult too to distinguish with certainty between the two groups by study of the histology of their spleens (Dacie 1943) The patients described by Citron (1922) Kaznelson (1924) Gripwall (1938) Thompson (1939) and Dacie (1943 Case 8) respectively as not responding to splenectomy appear in retro spect to be acquired cases

In Gripwall s case the family history was doubtful and his patient failed to respond to blood transfusion

Kaznelson s patient was a woman aged 58 with a recent onset of severe anæmia and no family history Splenectomy was followed by relapse as in Gripwall s patient the enlargement and great congestion of the liver at postmortem were striking features

Thompson s patient was a young woman suffering from jaundice of brief duration and considerable intensity Anæmia subsided after splenectomy only to reappear again after a few days The patient died 3 months later severely anæmic and with a reticulocyte count of 90% At postmortem many accessory spleens measuring 1–8 cm in diameter were found in the left upper quadrant of the abdomen There was no mention of any family history of hæmolytic anæmia and although the presence of accessory spleens provided an explanation for the relapse (if the patient was in fact suffering from hereditary spherocytosis) the acute onset and extreme severity of the disease the very transitory improvement following splenectomy and the lack of a family history all point to the diagnosis of acquired hæmolytic anæmia

West Watson and Young s case (1938) was associated with an ovarian cyst its removal resulted in dramatic improvement—splenectomy had previously proved ineffective (see also p 348)

(c) Patients possibly suffering from true Hereditary Spherocytosis Although some writers (e g McLaughlin 1942 Young 1947) mention that the presence of accessory spleens may occasionally be a reason for splenectomy failing to result in clinical cure there are very few wholly satisfactory reports of this in the literature

McLaughlin (1942) mentioned a patient who relapsed 2 years after splenectomy and died 5 years later At autopsy large hyperplastic lymph nodes 1–3 cm in diameter were found in the abdomen McLaughlin remarked that this was by no means as frequent a cause of recurrence as overlooked accessory spleens The diagnosis of this case is far from clear

Curtis and Movitz (1946) have also reported a possible example Their patient was a child aged 4 years who underwent splenectomy in 1933 for an acute hæmolytic episode There was said to be a family history of hæmolytic anæmia but no details were given The patient was well for the 4½ years following operation Then anæmia and reticulocytosis returned Laparotomy was carried out and two small accessory spleens were removed Gradual recovery ensued and the child was reported to be well two years later Again the exact diagnosis is obscure There was no mention of spherocytosis on the other hand highly phagocytic clasmatocytes were reported to be engorged with

splenectomy on the osmotic fragility of fresh blood also recorded mechanical fragilities and the osmotic fragility of incubated blood after splenectomy The results of both types of tests remained abnormal After splenectomy the mechanical fragility of fresh and incubated blood was slightly less than in patients before splenectomy but the increase in osmotic fragility on incubation was slightly greater in the splenectomized compared with the non splenectomized patients

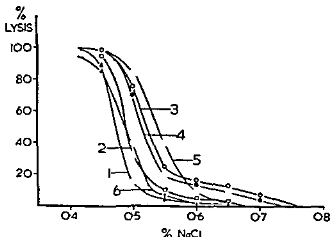


FIG 3 Changes in the osmotic fragility of the peripheral blood following splenectomy in a typical case of hereditary spherocytosis (redrawn from Dacie 1943) (1) Before anæsthetization ( ) after induction of anæsthesia (3) blood from splenic vein (4) at completion of operation (5) 24 hours after operation and (6) five days after operation

Dacie (1943) studied osmotic fragility changes in detail In seven patients he observed transient increases in fragility 24 hours after operation in two out of three patients examined the proportion of markedly fragile erythrocytes in the peripheral blood previously present in small numbers and responsible for the tail of the fragility curve was found to have increased by the time the operation had been completed and to increase still further during the next 24 hours (Fig 3) The increase at the time of operation was thought to be due to manipulation and compression of the spleen forcing very fragile corpuscles into the general circulation The further increase in fragility which developed within the next 24 hours was attributed to a progressive increase in the spherocytosis of these already markedly fragile cells Before splenectomy such very fragile cells would almost certainly have been removed from the general circulation by the spleen Quantitative

✓ The Effects of Splenectomy

It has already been mentioned that clinical cure is the almost invariable if not the invariable result of splenectomy in hereditary spherocytosis Nevertheless hæmatological signs of the disease persist and very interesting changes take place at the time of operation and during convalescence

Changes in Erythrocytes and Hæmoglobin *Detailed* studies of the changes in erythrocyte counts and hæmoglobin levels during and immediately after splenectomy were made by Doan Curtis and Wiseman (1935) and Sharpe McLaughlin and Cunningham (1939) Sharpe McLaughlin and Cunningham carried out serial blood counts at short intervals on eight patients and found that on an average the erythrocyte counts rose by about 900 000 cells per c mm in the early stages of the operation whilst the spleen was being handled prior to its actual removal The increase in erythrocyte count continued but not so sharply for 1 to 2 hours after removal of the spleen and then gradually *subsided becoming close to the pre operative level at about* 48 hours after operation Thereafter a gradual rise took place the counts reaching 5 000 000 cells per c mm in about a month One patient had a transient polycythæmia the erythrocyte count being 7 200 000 cells per c mm three months after splenectomy Sharpe McLaughlin and Cunningham (1939) made the additional point that the rises in erythrocyte counts at the time of operation did not occur to anything like the same extent in atypical hæmolytic anæmia Presumably the relatively great rise in the counts in typical hereditary spherocytosis is the consequence of the extraordinary degree of congestion of the spleen in that disease Sharpe and his co workers also reported dramatic temporary increases in leucocyte counts maximal in four to eight hours the rises were much less marked in their atypical cases It is generally agreed that the late results of splenectomy are excellent and that the patients erythrocyte counts and hæmo globin levels remain within the normal range for the rest of their *lives The author s own data are summarized in Table 3 (p 57)*

Osmotic Fragility after Splenectomy The effect of splenectomy on erythrocyte osmotic fragility has been repeatedly studied Most authors have reported a moderate increase in resistance after operation but rarely a return to normal The early literature was reviewed by Meulengracht (1922) and some more recent observations were listed by Dacie (1943) Young Izzo and Platzer (1951) in addition to studying the effect of

life *Target cells* observed in normal subjects and others after splenectomy however are not usually recognizable in post splenectomy films of the blood of patients with hereditary sphero cytosis—presumably this change is prevented by the persisting tendency to spherocytosis *Siderocytes* occur after splenectomy in variable numbers in the peripheral blood (Table 2 p 21) One patient was especially interesting for large numbers of siderocytes appeared as a transient phenomenon in the peripheral blood shortly after splenectomy Within a few weeks they had practically disappeared (Douglas and Dacie 1953) Another feature of post splenectomy films is an increased tendency to crenation This seems to be commonly found after splenectomy irrespective of the reason for the operation

Reticulocyte Counts after Splenectomy It is generally believed that reticulocyte counts fall to normal after splenectomy in patients with hereditary spherocytosis Young Izzo and Platzer (1951) reported an average of 0 85% reticulocytes with a range from 0 2 to 2 1% in twelve patients all examined a year or more after splenectomy There are however a few records of reticulocyte counts remaining slightly raised Dacie (1943) found that in six of twelve patients examined between 3 months and 6 years after splenectomy the counts ranged from 1 5 to 3 6% The counts in 20 patients are recorded in Table 3 the mean count was 1 4% and in three patients the count exceeded 2 5% Counts exceeding 2 5% have also been reported by Gripwall (1938) Thompson (1939) and Singer Miller and Dameshek (1941) It is fair to say however that great care in counting is necessary after splenectomy for it is difficult to differentiate with certainty cells containing very small amounts of reticulo filamentous material from siderocytes containing Pappenheimer bodies which also stain with cresyl blue

Bile Pigment Metabolism after Splenectomy The plasma bilirubin concentration falls significantly within a few days of splenectomy Whether or not the level falls to within the normal range in every case is not quite clear Meulengracht (1938) mentioned early reports of mild recurrences of jaundice after operation and Singer Miller and Dameshek (1941) gave values between 0 9 and 1 1 mg in 4 patients More recently Edwards (1951) reported levels between 0 8 and 1 7 mg per 100 ml in twelve patients On the other hand Young Izzo and Platzer (1951) found strictly normal values (0 1 to 0 8 mg per 100 ml) in twelve patients one or more years after splenectomy The author s own observations on 10 patients after splenectomy ranged between

curves at 24 hours after operation showed no appreciable alteration in the point of initial lysis (the saline concentration causing 1% lysis) By the third day after splenectomy a reduction in median fragility was evident and the point of initial fragility had shifted a little towards the normal indicating a disappearance of the most fragile cells During the latter half of the first week the median osmotic fragility of all the patients moved back towards the pre operative level the tails of the curves having by then largely disappeared By the tenth day the shape of the curves was generally of the normal almost vertical type with the median fragility at about the pre operative level The curves remained at about this level thereafter

The regular loss of the tails of fragile cells after splenectomy suggests that before operation the tails are produced by erythrocytes which at one time trapped in the spleen and made more spherocytic have escaped again into the peripheral circulation Loss of the tails of fragility curves after splenectomy was also reported by Waugh and Lamontagne (1940) in one case and more recently by Young Izzo and Platzer (1951) Young Platzer Ervin and Izzo (1951) also observed transient increases in fragility immediately following splenectomy in one well studied case

In patients whose fragility curves lack tails and are more nearly normal in shape little or no reduction in fragility may result from splenectomy in Cases 2 and 3 (p 78) for instance the initial fragility was even slightly increased following removal of the spleen The M C F too may slightly increase following splenectomy even in patients in whom the initial fragility is markedly decreased In 7 recent cases the mean M C F was 0 461% NaCl before splenectomy and 0 470% NaCl after splenectomy The same observation has been made by Young and Miller (1953)

There are in fact probably two factors at work acting in different directions which affect osmotic fragility after splenectomy—the removal of the spleen which when *in situ* makes the erythrocytes which traverse it more fragile and the longer survival of the patient s erythrocytes in his circulation as the consequence of splenectomy As the fragility of a spherocyte probably increases the longer it survives this tends to counter balance the effect of the removal of the spleen

Morphology of the Erythrocytes after Splenectomy It is generally agreed that the microcytosis and spherocytosis persist after splenectomy although there is some return towards the normal as the result of the loss of the most markedly spherocytic cells (Hawksley 1936 Vaughan 1937 Meulengracht 1938) This like the loss of the tails of fragile cells in osmotic fragility curves can be attributed to the absence from the circulation of erythrocytes previously trapped in the spleen and made more fragile thereby

As after splenectomy in normal subjects or in people suffering from other diseases *Howell Jolly bodies* begin to appear in the peripheral circulation a day or so after splenectomy thereafter they may be found in small numbers for the rest of the patient s

right in regard to acquired hæmolytic anæmia, but probably wrong about the hereditary type

In the last ten years fresh evidence has been accumulated which is strongly in favour of the hypothesis of an intrinsic corpuscular defect This evidence has been both direct and indirect trans fusion experiments provided direct evidence for the intrinsic nature of the corpuscular defect by showing that the corpuscles of the patient underwent rapid hæmolysis in a normal recipient and indirect evidence by demonstrating that normal corpuscles survived normally in patients suffering from hereditary sphero cytosis Dacie and Mollison s (1943) experiments moreover showed conclusively (*a*) that normal erythrocytes *were not* destroyed by the enlarged spleen of hereditary spherocytosis and (*b*) that spherocytes *were* destroyed by normal spleens This work since confirmed by other workers indicated that the enlargement of the spleen was secondary rather than primary and that it was acting as a destroyer of abnormal corpuscles

The Nature of the Corpuscular Defect The fact that spherocytosis is a progressive process has already been remarked upon As the corpuscle circulates it becomes more spheroidal with a progressively decreasing surface area It is probable that this process is accelerated when blood is stagnant within the spleen The high hæmoglobin concentration of the spherocyte its slightly diminished potassium content and its possibly low surface lipoid content have also been mentioned (p 64)

The demonstration of increased corpuscular osmotic fragility *in vitro* was never a satisfactory explanation for lysis *in vivo* for there never seemed any likelihood that the tonicity of the plasma would be diminished in any organ of the body sufficiently to cause osmotic hæmolysis The observations of Ham and Castle (1940a and b) and of Dacie (1941) on the rapid spontaneous hæmolysis of spherocytes were particularly significant for they provided the first satisfactory demonstration of an abnormal tendency to lysis *in vitro* which might be applicable to conditions *in vivo* The subsequent demonstration of the increased sensitivity of spherocytes to mechanical trauma was less significant for in the absence of the spleen spherocytes clearly withstand the wear and tear of circulation very well indeed as the clinical results of splenectomy demonstrate

Selwyn and Dacie (1954) have shown that the cation changes which occur when spherocytes are incubated *in vitro* in serum are not grossly abnormal and do not seem to be correlated with or responsible for the rapid lysis This appears more likely to be

0 3 and 1 6 mg with a mean (0 74 mg) towards the upper limit of the normal range (Table 3)

There are few relevant observations on the fæcal excretion of urobilinogen after splenectomy Goldschmidt Pepper and Pearce (1915) *found that the output of pigment fell after splenectomy to about one* tenth of its previous level becoming very close to the normal Eppinger (1920) reported a decrease in excretion after splenectomy to one quarter of the pre splenectomy level but the excretion still remained above the normal More recently Watson (1937) reported values within the normal range in 4 patients after splenectomy In 1 patient a figure of 616 mg per day was observed 15 days after operation however the output was normal (85 mg) 2 months later Barker (1938) reported normal figures in 3 patients after splenectomy and Singer Miller and Dameshek (1941) subnormal values for the hæmolytic index in 3 patients after splenectomy and a normal value in 1 patient

PATHOGENESIS OF HEREDITARY SPHEROCYTOSIS

From the very first most writers have linked the presence of excessive hæmolysis with an abnormality of the patient s erythrocytes Vanlair and Masius (1871) for instance quite correctly suggested that the microcytes they observed were senile erythrocytes on the way to destruction (globules atrophiques) and compared the microcytosis with that caused by heating blood Chauffard s (1907) discovery of the increase in osmotic fragility (and his rediscovery of the microcytosis) led to the concept of fragilité globulaire as the cause of the hæmolysis *in vivo* Widal and his associates (Widal and Philibert 1907) believed that the abnormal corpuscular fragility was the primary factor and sufficient in itself to bring about hæmolysis by the normal hæmolytic processes of the body Troisier (1910) complicated the issue by attributing the erythrocyte fragility to the fixation on the corpuscles of hæmolysins Banti (1913) elaborated this concept still further and postulated that the spleen was an important source of hæmolysin formation

The view that hæmolysis depended upon a primary abnormality of the erythrocytes has had many adherents (Naegeli 1931 Haden 1934 Thompson 1936 Vaughan 1936 Gripwall 1938 etc) Meulengracht (1938) on the other hand after careful weighing of the evidence favoured *a hyperactive condition of* the spleen as the primary and fundamental factor Dameshek and Schwartz s (1938) experiments with hæmolytic immune sera resuscitated Troisier s idea of erythrocyte damage due to hæmolysins As will be discussed later Dameshek and Schwartz were

which eventually lead to hæmolysis may well take place *in vivo* in blood stagnant in the spleen

The evidence for the stagnation of blood within the spleen in hereditary spherocytosis is admittedly circumstantial However inspection of a section of a spleen with its pulp cords typically grossly engorged

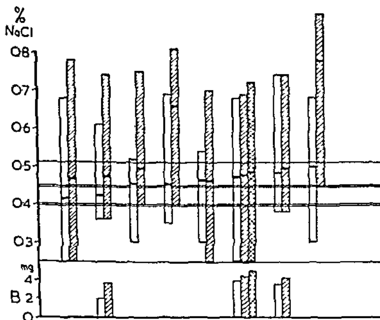


Fig 36 Differences between the osmotic fragilities and serum bilirubin concentrations (B) of splenic vein blood and peripheral blood *in cases of hereditary spherocytosis* The splenic vein samples were taken from the large vein draining the spleen as soon as possible after surgical excision Open rectangles represent peripheral blood samples hatched rectangles represent splenic vein samples the cross hatched sample was obtained from the splenic pulp See also Fig 31

with blood leads to the conclusion that there is so much blood present that it would be impossible for it all to escape quickly from the spleen labyrinth into the relatively small venous channels Support for this view was obtained by the author when he found that it took far longer to wash the spleens from patients with hereditary spherocytosis free from blood by saline perfusion through the splenic artery than in *comparable* experiments carried out with control spleens This seemed likely to be due to the fact that the spleen of hereditary spherocytosis contained so much more blood initially Dacie (1943) however found

the consequence of a membrane defect which leads to a relatively rapid irreversible contraction of the surface of the cell The rapidly progressive increase in osmotic fragility on incubation is the result of the unusual degree of cell membrane contraction

It appears possible that the abnormality of the spherocyte lies in an acceleration due to some congenital defect, of a normal degenerative process affecting the cell membrane of the erythrocyte which takes place particularly under conditions of stasis The nature of the membrane change and of the processes involved in its degeneration remains obscure Dacie (1941) observed that lysis was slowed but not restored to normal when spherocytes were placed in serum inactivated at 56° C, and several authors (Gripwall 1938 Fahraeus 1939) have attributed lysis in vivo as well as in vitro to the action of lysolecithin formed in stagnant blood as the result of enzyme activity (Bergenhem and Fahraeus 1935 Bergenhem 1939) This work however must be considered unproven

Recently Prankerd Altman and Young (1954) have reported observations which suggest that the erythrocyte carbohydrate metabolism may be abnormal in hereditary spherocytosis They found using a tracer technique employing radio phosphorus that although the uptake of phosphorus was normal more inorganic phosphate was formed relative to 2 3 diphosphoglyceric acid and adenosine triphosphate a relationship which was the reverse of the normal finding

The Hæmolytic Action of the Spleen Direct evidence for the hæmolytic activity of the spleen is provided by observations on the bilirubin content of splenic vein blood Values considerably higher than in the peripheral blood have been found (see Gripwall 1938 and Fig 36) Similarly the osmotic fragility of blood from the splenic veins or more particularly from the spleen pulp has been found to be greater than that of blood taken from the peripheral circulation (MacAdam and Shiskin 1922 Campbell and Warner 1925–26 Gripwall 1938 Young Platzer Ervin and Izzo 1951 Weisman Hurley Harris and Ham 1953, and Fig 36) There can be no doubt therefore that the osmotic fragility of the erythrocytes is increased in some way by their passage through the spleen There is evidence which suggests that the circulation of blood through the spleen in hereditary spherocytosis is slow (see below) It seems likely that the slowness of the circulation is of major pathogenetic importance for the changes which occur in blood incubated at 37 C in vitro and

Whipple considered that whereas normal discoidal cells probably could circulate through the spleen without difficulty spherocytes because of their shape might find it difficult to traverse the slit like stomata leading from the pulp into the splenic sinuses This hypothesis is attractive but difficult to confirm or refute It assumes that the erythrocytes lie mostly in the pulp during life

The last point to be considered is the way in which the spleen brings about erythrocyte destruction As compared with spleens removed from patients with acquired hæmolytic anæmia erythrophagocytosis is not conspicuous It seems likely to the author and to Young Platzer Ervin and Izzo (1951) that lysis of the stagnant blood takes place by the same mechanisms which produce lysis of incubated blood *in vitro* As already mentioned this cannot be satisfactorily explained by accumulation of osmotically active metabolites which cause swelling and eventually osmotic lysis Degenerative changes particularly affecting the cell membrane seem to be the more likely explanation It is uncertain whether or not the degeneration can be explained by an accumulation in the stagnant blood of potentially hæmolytic substances such as lysolecithin or other tissue lysins (see Ponder 1951) to which hereditary spherocytes are possibly peculiarly sensitive Attempts to demonstrate an increased formation of lysolecithin in spleen blood have been unsuccessful (Singer 1941) It is equally possible but unproven that the lysis is due to intrinsic abnormal or unbridled metabolic activity within the cell membrane and that hæmolysis *in vivo* is accelerated as it is *in vitro* by a fall in the glucose concentration in the stagnant blood (Selwyn and Dacie 1954) Be the exact mechanism what it may it now seems clear that hæmolysis within the spleen is due to the spleen s recognition of the abnormal nature of the spherocytes with consequent sequestration of the cells within the spleen and lysis there in consequence of the cells peculiar sensitivity to the effects of circulatory stagnation

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that large oval fowl cells could transverse the spleen (in small numbers) as rapidly as in the normal and concluded that there was no anatomical block in the circulation through the spleen It seemed likely never theless that much of the pulp was a backwater outside the main current of the blood stream

If it is conceded that congestion and stagnation of blood within the spleen is the rule in hereditary spherocytosis it remains to be decided how the congestion is brought about and how stagnation within the spleen leads to the destruction of the erythrocytes It has to be borne in mind that splenic congestion is also very frequently found in other types of hæmolytic anæmias as well as in hereditary spherocytosis Thus congestion with blood may be the most striking pathological change in acquired hæmolytic anæmia (Dameshek and Schwartz 1940) it is also characteristically found after the administration of hæmolytic sera or poisons to laboratory animals (Banti 1913 Eppinger 1920)

It seems likely that the congestion is due to the spleen s remarkable property of filtering off from the blood stream damaged or abnormal corpuscles irrespective of whether the damage is due to the effects of immune antibodies hæmolytic poisons or whether the erythrocytes are inherently abnormal as in hereditary spherocytosis Recently it has been shown experimentally by differential agglutination that the spherocytes of hereditary spherocytosis are more easily trapped in the spleen than are normal corpuscles (Emerson Shen and Castle 1946 Emerson Shen Ham and Castle 1947 Young 1947 Young Platzer Ervin and Izzo 1951 Weisman *et al* 1953) Of particular significance is the observation of Young and his colleagues that spleens excised from patients with thrombocytopenic purpura were also capable of selectively trapping spherocytes when perfused *in vitro* with a mixture of spherocytes and normal cells This is in accord with Dacie and Mollison s (1943) observation of the rapid elimination from the circulation of spherocytes transfused to normal subjects and Schrumpf s (1951) contrasted observation of the relatively good survival of spherocytes transfused to a previously splenectomized but otherwise normal recipient

The problem as to how the congestion in the spleen is brought about has not been solved Various hypotheses have however been submitted Klemperer (1938) suggested that the presence of abnormal corpuscles reflexly initiated arterial vasodilatation and Whipple (1941) postulated that the stagnation was due to the abnormal shape of the corpuscles i e the spherocytosis

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CHAPTER 3

THE CONGENITAL HÆMOLYTIC ANÆMIAS

II HEREDITARY ELLIPTOCYTOSIS AND ELLIPTOCYTIC HÆMOLYTIC ANÆMIA

HEREDITARY ELLIPTOCYTOSIS

The presence of elliptical erythrocytes in man was first described by Dresbach in 1904 in the blood of a mulatto Since then the condition has been observed in many races throughout the world and it now has an extensive literature

Bishop (1914) observed elliptocytes in the blood of a brother and sister belonging to the same family and it is now known that the abnormality is inherited as a Mendelian dominant (Wyandt Bancroft and Winship 1941) Recent work by Goodall Hendry Lawler and Stephen (1953) has shown that the gene determining the elliptocytosis is located on the same chromosome as that carrying the genes for the Rh blood group system

Morphology of Elliptocytes The degree of ellipticity of the erythrocytes varies not only from subject to subject but within the cell population of any particular person (Figs 37 and 43) Usually up to 90% of the cells are affected some being markedly elongated others merely oval The nucleated precursors of elliptocytes are round and reticulocytes are also round or at any rate conspicuously less elliptic than adult corpuscles

Some writers have classified elliptocytes on the basis of diameter measurements (e g Gunther 1928 Lambrecht 1938 Guasch and Raichs 1948 Zini and Leubner 1951) and the distinction between the abnormal trait and normal blood which may contain up to 10% of definitely oval cells has been carefully defined (Hedenstedt 1947) The mean cell volume of elliptocytes has usually been reported as normal The erythrocyte osmotic fragility is normal at least in patients without anæmia (Fig 31) Hæmoglobin concentration is also normal and no abnormality affecting the hæmoglobin itself has so far been demonstrated It is interesting to note however that the elliptocytosis is not fully evident at birth but increases to a maximum by about the end of the third month (Hunter 1932-3 Helz and Menten 1944)

Erythrocyte counts above the normal have been observed occasionally in hereditary elliptocytosis Stephens and Tatelbaum (1934-35) reported for instance that the counts of the affected members of a family averaged 6 470 000 cells per c mm although their hæmoglobin levels were within the normal range

Most authors have looked upon hereditary elliptocytosis as a harmless trait and both Wyandt Bancroft and Winship (1941) and Hedenstedt (1947) concluded that there was no direct relationship between elliptocytosis and anæmia The number of patients now recorded suffering from overt hæmolytic anæmia and elliptocytosis (see p 96) suggests however that this conclusion is erroneous

Survival of Elliptocytes in vivo The life span of elliptocytes is obviously of great interest in connection with the possible connection between elliptocytosis and hæmolytic anæmia Early reports based on the results of transfusing blood containing many elliptocytes to normal recipients and the subsequent recognition of the elliptical cells in samples of the recipients blood withdrawn from time to time after the transfusion suggested that the survival of the elliptocytes was considerably shorter than normal (Vischer 1938–39 Kirkegaard and Larsen 1942) Later Hedenstedt (1947) reported that elliptic cells had an average half life of 13 1 days and that they disappeared from the recipient s circulation in an exponential manner A more recent paper by Berlin and Hedenstedt (1952) however has cast doubt on the validity of attempting to deduce the elimination of elliptocytes from the circulation by actually counting the elliptic cells According to Hedenstedt s photomicrographic method the elliptocytes disappeared exponentially in the recipient s circulation after 15 to 20 days one half of them could no longer be recognized in photographs Berlin s data based on counts made by the Ashby method on the same blood showed however that the half life of the elliptocytes was about 50 days In order to reconcile the two apparently contradictory sets of observations Berlin and Hedenstedt came to the conclusion that the elliptocytes were gradually transformed into rounded corpuscles in the normal environment This interpretation is possible if unexpected it certainly needs confirmation (see below)

Trinick (1948) transfused the blood of a healthy non anæmic blood donor 90% of whose corpuscles were elliptic in shape into a recipient recovering from a recent blood loss and found using the Ashby method that the survival of the donor s corpuscles was normal i e 100 to 110 days

More recently Motulsky Singer Crosby and Smith (1954) repeated the experiment of Berlin and Hedenstedt using the blood of three different donors Cell survival was studied by the Ashby method as well as by a visual method Contrary to the observations of Berlin and Hedenstedt roughly similar results were

obtained by both techniques In two cases the survival of the elliptocytes was normal that of the third donor was however abnormal (elimination complete in 45 days) It is interesting to note that in the third donor the existence of a slightly raised reticulocyte count and a serum bilirubin level at the upper limit of the normal range and an elevated hæmolytic index suggested that the donor herself was suffering from a mild well compensated hæmolytic process

Reticulocyte Counts in Hereditary Elliptocytosis There are only a *few studies available on the reticulocyte counts of healthy non anæmic* subjects with hereditary elliptocytosis Mason (1938) stated that the number of reticulocytes was not increased Wyandt and his co workers (1941) carried out counts on 14 cases in one patient with anæmia (see p 97) there were 13% reticulocytes in the non anæmic subjects the counts ranged from 1 5% to 3 6% Kirkegaard and Larsen (1942) reviewed the reports of reticulocyte counts in 21 cases Fifteen of the patients were not anæmic in ten the reticulocyte counts were said to be greater than normal

HEREDITARY ELLIPTOCYTIC HÆMOLYTIC ANÆMIA

It is apparent from a study of the literature on hereditary elliptocytosis that *the presence of the elliptocytes does not usually give rise to any symptoms or signs of anæmia* Exceptionally however an undoubted and sometimes severe hæmolytic syndrome develops Between these two extremes there are patients in whom a minor degree of excess hæmolysis probably occurs in these the only obvious sign may be a slight but persistent rise in *their reticulocyte counts* The *relative frequency of major* or minor degrees of excessive hæmolysis is not yet known It seems to vary between family and family (see below) Penfold and Lipscomb (1943) in reviewing 400 cases of elliptocytosis reported in the literature concluded that in 12% of them there were signs of increased hæmolysis

Case Reports of Elliptocytic Hæmolytic Anæmia

One of the first reported cases of overt hæmolytic anæmia associated with elliptocytosis seems to be that of Hijmans van den Bergh (1928) The erythrocyte osmotic fragility was normal but except for this and the presence of the elliptocytes the hæmatological findings and the patient s symptoms and physical signs were said to be typical of congenital hæmolytic anæmia

Grzegorzewski (1933) described a family in which the erythrocytes of 14 persons were elliptic Six of them were mildly anæmic and gave a history of being slightly jaundiced from time to time Their erythrocyte osmotic fragilities were reported to be increased



FIG 37 Photomicrograph of a blood film of a patient carrying the trait of hereditary elliptocytosis The patient was not anæmic and there were no signs of a hæmolytic process × 00

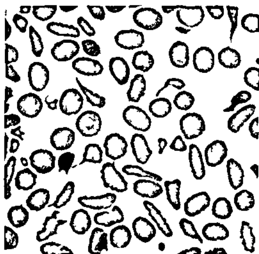


Fig 38 Photomicrograph of a blood film of a patient suffering from elliptocytic hæmolytic anæmia who had undergone splenectomy 14 years previously × 700 (Reproduced through the courtesy of Dr M C Verloop)

Mason (1938) described two most interesting examples of hæmolytic anæmia with ovalocytosis (elliptocytosis) The first patient was a white boy of 13 years who had experienced five attacks of anæmia in the preceding 8 years A large proportion (80%) of his erythrocytes were oval their osmotic fragility was normal His mother was not anæmic but in her blood film there were many oval cells His father s blood was normal but three children of a paternal aunt had died of an undiagnosed severe anæmia It seemed as if the patient s father might have been carrying a latent trait for anæmia which in his son converted a harmless elliptocytic trait into overt hæmolytic anæmia Mason s Case 4 was remarkable in that many of the elliptocytes were deformed and had tail like processes The patient s spleen had been removed 14 years previously this possibly had something to do with the morphological peculiarities (see Fig 38)

Lambrecht (1938) observed two instances of anæmia with jaundice and decreased osmotic resistance He considered that cases of elliptocytosis might be separated into active (hæmolytic) compensated and latent forms

Ciffin and Watkins (1939) studied three patients in one family who suffered from slight to moderate anæmia Elliptocytes and microcytes were present in their blood films Their serum bilirubin concentrations were increased (2 2 to 2 9 mg per 100 ml) and the erythrocyte osmotic fragilities were also slightly increased

Wyandt and co-workers (1941) reported the occurrence of one undoubted case of anæmia out of a total of 86 patients with elliptocytosis whom they investigated The affected patient was a child whose blood contained both elliptocytes and small spherocytic microcytes The blood of both parents contained typical elliptical erythrocytes neither was anæmic The more severe disease in their child may have represented therefore elliptocytosis in the homozygous state

Leitner (1943) studied seven members of the same family all with elliptical erythrocytes Two of them suffered from moderate anæmia with hyperbilirubinæmia reticulocytosis and a slight increase in osmotic fragility

Penfold and Lipscomb (1943) described elliptocytosis associated with hereditary hæmorrhagic telangiectasia Five of their patients were jaundiced two had palpable spleens

Holst Larsen s (1947) observations were remarkable especially for the variable intensity of the erythrocyte abnormality in his patients Unmistakable hæmolytic anæmia was present in three branches of a single family the elliptocytes being admixed with and seemingly merging into small microspherocytes and irregularly shaped microcytes in the more anæmic patients As a seriously affected case developed in three branches of the same family it is extremely unlikely that the severity could be explained by the gene being present in the homozygous state in each of the three branches

Lendval (1949) briefly described the incidence of severe anæmia in 3 brothers and sisters The parents were cousins typical elliptocytosis was transmitted from the maternal side on the paternal side 14 brothers and sisters died probably of hæmolytic anæmia This seems to be yet another example of the effect of the inheritance of traits for congenital anæmia from both sides of a family resulting in severe anæmia in children of the next generation

Gasser (1951) reported one patient with hereditary elliptocytosis (a girl aged 9 years) in whom he considered there was evidence for a mild compensated hæmolytic process

Dacie and his co workers (1953) described a remarkable instance of severe hæmolytic anæmia in a child who belonged to a family known to carry the elliptocytic trait The child was admitted into hospital when only 10 days old suffering from a rapidly increasing anæmia He was transfused and existed for the next 6 months on blood transfusions

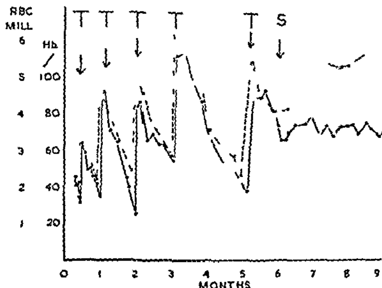


Fig 39 Changes in the erythrocyte count and hæmoglobin content of the blood of D H (Case II of Dacie *et al* 19.3) who under went splenectomy when six months old for an unusual type of elliptocytic hæmolytic anæmia

T = Transfusion • ———— • = hæmoglobin
S = Splenectomy x – – – – – x = erythrocyte count

Splenectomy was then carried out with immediate benefit Thereafter erythrocyte regeneration kept pace with hæmolysis (Fig 39) Post splenectomy blood films were remarkable for the marked variation in erythrocyte size and for the presence of numerous extremely small spherocytic microcytes and fragments of irregular shape (Fig 40) Erythrocyte osmotic and mechanical fragilities were markedly increased The blood of the child s father was normal but that of his mother and of a brother contained many oval or slightly elliptic cells (Fig 41) Neither his mother nor brother was anæmic The severe degree of the anæmia of the affected child is unexplained there was no obvious history of anæmia on the father s side of the family

The writer has investigated recently another example of elliptocytic hæmolytic anæmia through the courtesy of Dr Philip Ellman

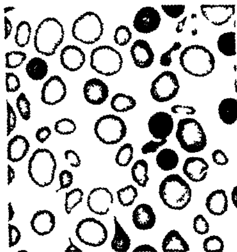


Fig 40 Photomicrograph of a blood film of D H (Case 11 of Dacie et al 1953). Many irregularly shaped spherocytes and small fragments of cells are present (after splenectomy) × 1 000

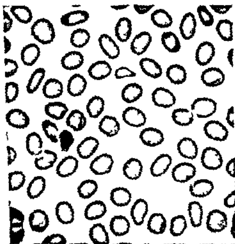


Fig 41 Photomicrograph of the blood film of Mrs H H mother of D H (Fig 40) There is a moderate degree of elliptocytosis. Mrs H was not anaemic × 700

[To face p 98

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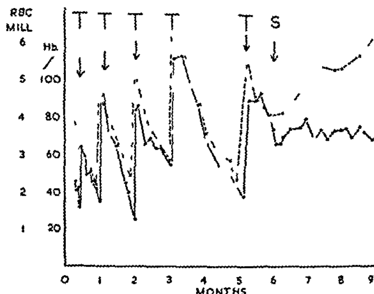


FIG 39 Changes in the erythrocyte count and hæmoglobin content of the blood of D H (Case 11 of Dacie *et al* 1953) who underwent splenectomy when six months old for an unusual type of elliptocytic hæmolytic anæmia

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Case Report Congenital Elliptocytic Hæmolytic Anæmia

Case 5 The patient a woman aged 35 was admitted into hospital in January 1952 suffering from pneumonia She made a good recovery from this illness but was found to be seriously anæmic and was in consequence transfused Her spleen was palpable From questioning it seemed likely that she had suffered from a mild anæmia and slight jaundice for many years previously but this had never been severe enough to cause symptoms As a young woman she had been an athlete of some distinction

Her blood has been examined on several occasions since her pneumonia Nearly all her erythrocytes are oval or moderately elliptic (Fig 42) The erythrocyte count has ranged between 3 400 000 and 3 900 000 cells per c mm and her hæmoglobin between 11 5 and 12 9 g per 100 ml The hæmoglobin concentration (33% to 36%) has been normal and the M C V slightly increased (99 to 109 c µ) Her reticulocyte count has varied between 11% and 17% and the plasma bilirubin level from 1 1 to 1 8 mg per 100 ml The erythrocyte osmotic fragility was normal

Her father and two children but not her mother seem to be carriers of an elliptical cell trait the degree of their elliptocytosis is however slight most of the erythrocytes being oval rather than elliptic in shape Neither the patient s father nor her two sons were anæmic and their reticulocyte counts bilirubin levels and osmotic fragilities were all normal A photomicrograph of the blood film of the patient s father is illustrated in Fig 43

Pathogenesis As already mentioned some authors have suggested that hereditary elliptocytosis should be looked upon as a harmless trait analogous to the sickle cell trait or the mildest forms of hereditary spherocytosis (Mason 1938 Kirkegaard and Larsen 1942 Penfold and Lipscomb 1943 Guarsch and Raichs 1948 Motulsky *et al* 1954) If this is a correct conception it remains to be explained how an apparently harmless trait in which excessive hæmolysis is usually absent or minimal and easily compensated for becomes converted occasionally into an overt hæmolytic anæmia The possibility that active hæmolysis only occurs when the trait is present in the homozygous form as is the case in Mediterranean anæmia (thalassæmia major) is not borne out by the facts Although both the parents of the anæmic patient described by Wyandt and co workers (1941) were shown to be bearers of the trait in most instances only one parent has been found to be affected It seems instead that the expressivity of the gene may be markedly modified in other ways than by the gain of an additional gene for elliptocytosis and that varying grades of increased hæmolysis are the result of this modification

In certain families several instances of hæmolytic anæmia have occurred This suggests the possibility that there are at

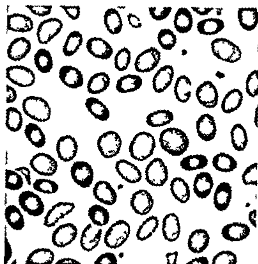


Fig 42 Photomicrograph of a blood film of Mrs La (Case 5) suffering from elliptocytic hæmolytic anæmia × 760

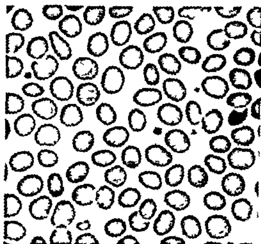


Fig 43 Photomicrograph of a blood film of Mr Lo the father of Case 5 (Fig 42) There is a slight degree of elliptocytosis Mr Lo was not anæmic and there was no evidence of a hæmolytic process × 700

length of time In Case 11 of Dacie and co workers (1953) the survival of transfused blood was proved to be normal the child being literally kept alive by transfusion Transfusion can therefore in all probability be confidently recommended as a palliative measure in the severely anæmic patient

Splenectomy There are a few reports in the literature on the effects of splenectomy in elliptocytic hæmolytic anæmia Most of the patients seem to have benefited but unfortunately in most instances only scanty details were given

Hijmans van den Bergh (19 8) reported that the jaundice of his patient receded Mason (1938) recorded the blood count of a patient (his Case 4) splenectomized 14 years previously as 4 230 000 erythrocytes per c mm and 72% hæmoglobin

Giffin and Watkins (1939) described good results in two cases after splenectomy with improvement in anæmia reduction in jaundice and less evidence of regeneration than before operation Holst Larsen (1947) mentioned the effect of splenectomy in two severely affected patients Although a substantial rise in hæmoglobin and loss of jaundice followed the operation the degree of elliptocytosis and spherocytosis was unaffected

Lendval (1949) reported improvement after splenectomy and stated that whereas during a hæmolytic phase (before splenectomy) erythrocyte thickness was increased after splenectomy only normal elliptocytes were present Another successful result was reported by Harrier and his colleagues (1952) Wilson and Long (1953) briefly reported the presence of hæmolytic anæmia and ovalocytosis in two elderly patients (a brother and his sister) Splenectomy carried out on one of them resulted in a cure of the anæmia and leucopenia After operation an increased number of ovalocytes and spherocytic microcytes was found in the circulating blood stream

The patient described as Case 11 by Dacie and co-workers (1953) derived striking benefit from splenectomy before operation the child s life depended on transfusion after operation erythrocyte formation more than kept pace with destruction (Fig 39) Whether patients whose erythrocytes have an increased osmotic fragility do better after splenectomy than those with normal fragility remains to be seen

Hereditary Elliptocytosis in Association with other Traits

It has already been mentioned that microspherocytosis and increased osmotic fragility may be observed in some patients suffering from elliptocytic hæmolytic anæmia In none of the case reports so far recorded does there seem however to have been conclusive evidence based on family studies for the presence of the trait of hereditary spherocytosis in addition to that of hereditary elliptocytosis Evidence for the association of elliptocytosis with the sickle cell trait (Pollock and Dameshek 1934 Fadem 1949) is likewise inconclusive

A few interesting examples are on record of other types of blood disease occurring in association with the trait for hereditary elliptocytosis the patient of Bang and Georg (1947) for instance may have

least two types of hereditary elliptocytosis a benign typical' form not associated with hæmolytic anæmia and a rarer type not infrequently associated with hæmolytic anæmia Although this is an attractive hypothesis there are at the time of writing insufficient data to warrant any clear cut differentiation

It is not possible to correlate the degree of erythrocyte abnormality and the incidence of hæmolysis On the one hand a severe degree of elliptocytosis is not necessarily accompanied by anæmia or signs of hæmolysis on the other hand overt hæmolytic anæmia as in Case 5 may be associated with only a moderate degree of elliptocytosis It seems probable however that some evidence of hæmolysis will always be found in those cases in which there is a tendency to form either elliptic or round spherocytic microcytes It does not appear likely that the presence of spherocytes (and increased osmotic fragility) necessarily indicates an admixture with the trait of hereditary spherocytosis it seems more probable that spherocytosis and microcytosis are one result of an increased expressivity of the gene for elliptocytosis This is well shown by Holst Larsen s (1947) patients by Case 11 of Dacie and co workers (1953) and by Wyandt and his colleagues (1941) patient in whom there was reason to believe that the elliptocytic trait might be present in the homozygous state However an increased rate of hæmolysis is not necessarily associated with spherocytosis and increased erythrocyte osmotic fragility The osmotic fragility was for instance normal in the patients described by Hijmans van den Bergh (1928) and by Mason (1938) and in the patient (Case 5) referred to on p 99

There is some evidence that the erythrocytes in hereditary elliptocytosis differ from the normal not only in morphology but in other ways also Selwyn (1953) studied the cation changes when blood was incubated *in vitro* and the effect of glucose on these changes and on spontaneous hæmolysis The results were normal except that glucose had less than its normal effect on reducing the amount of autohæmolysis This was so in three out of four non anæmic carriers of the trait as well as in two patients with active hæmolytic anæmia

Treatment of Hereditary Elliptocytic Hæmolytic Anæmia

Blood Transfusion There is practically no information on the survival of normal erythrocytes after transfusion to patients suffering from elliptocytic hæmolytic anæmia but what evidence there is suggests that normal corpuscles survive for the normal

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suffered from paroxysmal nocturnal hæmoglobinuria and that of Druez (1952) from an acquired hæmolytic anæmia of the auto immune type Other examples of an auto immune hæmolytic process being super imposed on a congenital hæmolytic anæmia are referred to on p 63

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rences in the type of anæmia in the two families A notable feature of the anæmia affecting the first family was the rapidity of autohæmolysis in vitro in the second family the most notable feature was the striking punctate basophilia of the erythrocytes

Crosby's (1950) report dealt with a large American family of mixed English and French antecedents in which a relatively mild chronic normocytic hæmolytic anæmia was found in seven (possibly in nine) out of 36 members Brachyphalangia was also found but this was not necessarily associated with anæmia One patient was investigated in detail Splenectomy was carried out but without benefit to his anæmia Morphologically his erythrocytes were biconcave discs occasional cells were oval or tailed a very few were spherocytes or target cells The erythrocyte mechanical fragility was normal but autohæmolysis on incubation was accelerated The patient's corpuscles survived only 12 days when transfused to a normal recipient An interesting additional abnormality was the presence of porphobilinogen in his urine on several occasions—he had however no definite symptoms or clinical signs of porphyria (see p 111)

Kaplan and Zuelzer (1950a) found a hæmolytic anæmia in three out of the 6 children of a family of French Canadian extraction Each child suffered from a moderately severe and slightly macrocytic anæmia There were no target cells or spherocytes but about half of the corpuscles were slightly or moderately oval The osmotic and mechanical fragilities were normal The erythrocytes of one of the patients were transfused to a normal recipient their survival was significantly shortened Splenectomy was not carried out It is possible that the children inherited the disease from their mother as about 15% of her corpuscles were slightly oval and she was mildly anæmic However the mother's reticulocyte count was within the normal range As the degree of ovality of the patients erythrocytes was far less than in typical hereditary elliptocytosis Kaplan and Zuelzer did not consider that there was any relationship between the anæmia from which the patients were suffering and hereditary elliptocytosis

Kaplan and Zuelzer (1950b) in another publication reported observations on two further children of Italian and American origin who also were affected with non-spherocytic hæmolytic anæmia Their anæmia was normocytic in type and there were occasional microspherocytes the osmotic resistance was however increased The patients corpuscles were relatively rapidly eliminated from the circulation of normal recipients Splenectomy resulted in slight improvement only The elder of the two children developed a transient acute hæmolytic episode with marked microspherocytosis and hæmoglobinuria apparently due to the formation of auto-antibodies During and shortly after this hæmolytic episode the survival of transfused normal corpuscles was impaired Later transfused normal corpuscles survived normally

Another patient suffering from an apparently congenital hæmolytic anæmia was described by Feinberg and Watson (1951) The patient was a negro his eight brothers and sisters and his two children appeared to be unaffected His anæmia was normochromic and slightly macrocytic and a striking feature of his blood film was the large number of stippled cells present Osmotic fragility before and after incubation at 37° C was normal and tests for sickling were repeatedly negative A splenic aspiration was carried out smears showed fewer

CHAPTER 4

THE CONGENITAL HÆMOLYTIC ANÆMIAS

III CONGENITAL NON-SPHEROCYTIC HÆMOLYTIC ANÆMIAS AND UNCLASSIFIED TYPES

CONGENITAL NON SPHEROCYTIC HÆMOLYTIC ANÆMIA

UNDER this title will be grouped together forms of congenital hæmolytic anæmia which differ fundamentally from hereditary spherocytosis In these atypical cases the anæmia is generally macrocytic there is often a moderate degree of ovalocytosis and sometimes conspicuous punctate basophilia Spherocytes are not present and osmotic fragility is characteristically normal Splenectomy is not followed by *permanent clinical cure* This type of disease is rare but probably not as rare as the few reports in the literature would suggest

Clinical histories and hæmatological findings of patients probably suffering from congenital non spherocytic hæmolytic anæmia have been recorded by Thompson (1939) Haden (1947) Crosby (1950) Kaplan and Zuelzer (1950a and b) Feinberg and Watson (1951) Dacie Mollison Richardson Selwyn and Shapiro (1953) Holliday (1953) and Lipton Grossman and Richmond (1953) *As it is probable that not all these patients suffered from exactly* the same disorder the salient features of the case reports referred to above will be considered individually

Case Reports in the Literature

Thompson (1939) referred briefly to three families suffering from congenital hæmolytic anæmia with normal erythrocyte osmotic fragilities He mentioned that splenectomy had been carried out in several of the patients without improvement

Haden (1947) described two families one American and the other Hungarian affected with a new type of hereditary hæmolytic jaundice In the first family three members of two generations were affected in the second family four members of three generations In both families the anæmia was macrocytic in type osmotic fragilities were normal and there was no spherocytosis Splenectomy was carried out on one patient but this did not alter the course of the disease Haden s description indicates that although both families were affected by a non spherocytic hæmolytic anæmia there were important diffe

rences in the type of anæmia in the two families A notable feature of the anæmia affecting the first family was the rapidity of autohæmolysis *in vitro* in the second family the most notable feature was the striking punctate basophilia of the erythrocytes

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stippled cells than in the peripheral blood Feinberg and Watson concluded that the spleen was either destroying the stippled cells or sifting out the inclusions from the stippled cells This disorder seems to be very similar to that affecting the second of Haden s families

Holliday (1953) described a family in which at least four members suffered from a non spherocytic hæmolytic anæmia Basophilic stippling of the erythrocytes was conspicuous in three of the patients

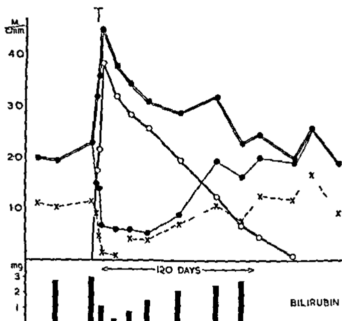


FIG 44 Hæmatological changes after a large blood transfusion given to a patient suffering from congenital non-spherocytic hæmolytic anæmia (Case 1 of Dacie *et al* 1953)

● ═══ ● represents the total erythrocyte count
o ——— o the count of donor erythrocytes and
● ——— ● the recipient s erythrocyte count
x - - - - - x represents the absolute reticulocyte count
The serum bilirubin concentration is represented by the upright rectangles at the bottom of the figure

One patient was studied in considerable detail mechanical fragility was found to be slightly increased and the patient s erythrocytes were reported to undergo more rapid autohæmolysis both at 4 C and at 37 C when suspended in sterile isotonic saline than did normal corpuscles In plasma however the rate of autohæmolysis was normal

Lipton Grossman and Richmond (1953) described the clinical and hæmatological data in two sisters who probably suffered from a congenital non spherocytic hæmolytic anæmia The early results of splenectomy were encouraging although anæmic the children

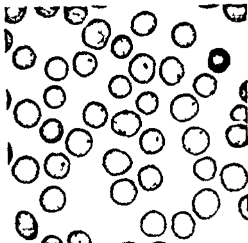


Fig 4 Photomicrograph of a blood film of a patient suffering from a congenital non-spherocytic hæmolytic anæmia (Case 1 of Dacie *et al* 1953) Splenectomy had been carried out more than [illegible] years previously Numerous Pappenheimer bodies are present × [illegible]00

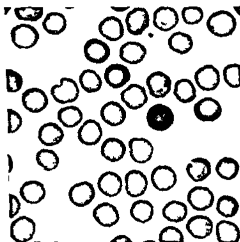


Fig 46 Photomicrograph of a blood film of a patient suffering from a congenital non-spherocytic hæmolytic anæmia (Case 2 of Dacie *et al* 19[illegible]3) Splenectomy had been carried out [illegible] months previously × [illegible]00

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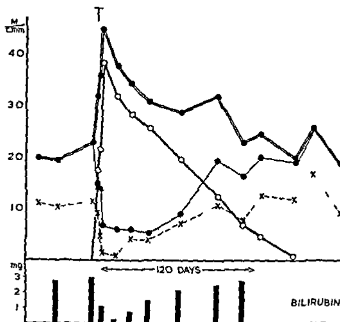


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managed to compensate for hæmolysis after operation without transfusions being necessary

Dacie and colleagues (1953) described 4 patients with congenital non spherocytic hæmolytic anæmia belonging to different families all had undergone splenectomy without their anæmia being alleviated

Case 1 a woman aged 29 years had had her spleen removed in early childhood Her erythrocytes (after splenectomy) were mostly macrocytes rounded in contour and nearly all contained Pappenheimer bodies (Fig 45) Osmotic fragility was slightly diminished Hæmolysis *in vivo* was evidently greatly accelerated for her reticulocyte count constantly exceeded 60% The normal survival of transfused normal corpuscles is shown in Fig 44 A notable feature was that her blood underwent spontaneous hæmolysis *in vitro* at more than 10 times the normal rate (see p 111)

Case 2 was a boy aged 7 years whose spleen had been removed 6 months previously without substantially influencing the course of his disease His erythrocytes (after splenectomy) were mostly rounded in contour and slightly macrocytic (Fig 46) Osmotic fragility was slightly increased

Case 3 a boy aged 17 had undergone splenectomy when 14 years of age without the operation influencing the course of his disease Before splenectomy many of his erythrocytes were macrocytes some were slightly oval in shape In addition occasional pear-shaped poikilocytes and small contracted corpuscles were present After splenectomy target cells were conspicuous (Fig 47) Osmotic fragility was normal before splenectomy and slightly diminished afterwards

Case 4 a girl aged 13 years had had her spleen removed 6 years previously Hæmolysis was still proceeding at a rapid rate Her erythrocytes (after splenectomy) were mostly macrocytes with a round contour containing conspicuous Pappenheimer bodies (Fig 48) This case seemed to be almost identical with Case 1

Dacie and co-workers (1953) also described a fifth patient (Case 5) suffering from a congenital non spherocytic hæmolytic anæmia His spleen had not been removed at the time of their report He was a boy aged 15 years only moderately anæmic but always visibly jaundiced with a plasma bilirubin level usually in the region of 4 to 5 mg per 100 ml His erythrocytes were slightly macrocytic with a definite tendency to ovalocytosis (Fig 49) His mother appeared to have the trait in a very mild form (Fig 50)

Further details of this patient can now be given as clinically obvious jaundice was continuously present it was thought advisable to carry out splenectomy even though the chances of any marked improvement seemed remote The spleen was moderately enlarged it was found to weigh 260 g when allowed to empty itself of blood (about twice the normal weight for the patient s age) Histological examination showed less congestion with blood than in hereditary spherocytosis (p 70) The Malpighian bodies were normal in size the pulp cords were unusually prominent and contained moderate numbers of erythrocytes The littoral cells of the sinuses were conspicuous and iron containing pigment was present in moderate amounts

No substantial benefit resulted from removal of the spleen The hæmoglobin level ranged between 11 3 and 12 7 g per 100 ml during the first year after operation and the reticulocyte count has varied

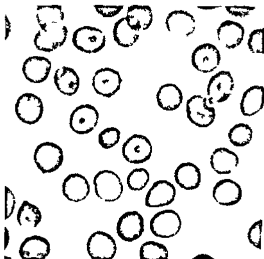


Fig 47 Photomicrograph of a blood film of a patient suffering from a congenital non spherocytic hæmolytic anæmia (Case 3 of Dacie *et al* 1953) Splenectomy had been carried out four years previously Target cells are conspicuous × 700

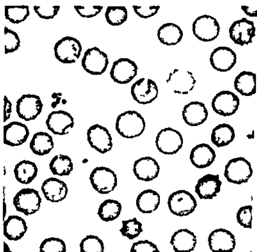


Fig 48 Photomicrograph of a blood film of a patient suffering from a congenital non spherocytic hæmolytic anæmia (Case 4 of Dacie *et al* 1953) Splenectomy had been carried out eight years previously × 700

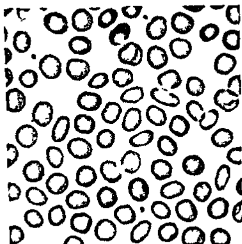


Fig 49 Photomicrograph of a blood film of a patient suffering from a congenital non-spherocytic hæmolytic anæmia (Case 5 of Dacie *et al* 1953) Before splenectomy × 700

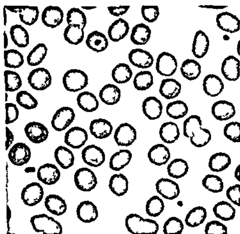


Fig 50 Photomicrograph of a blood film of Mrs S the mother of Case 5 of Dacie and co-workers (1953) × 700

[T f *p* 108

between 4 7 and 7 4% as compared with pre operative hæmoglobin levels and reticulocyte counts of 11 0 to 11 5 g and 4 to 6% respectively On the other hand the serum bilirubin level has been slightly lower averaging 2 7 mg per 100 ml between 3 and 12 months after operation compared with a pre operative average figure of 4 4 mg per 100 ml

As will be discussed under *Pathogenesis* (p 109) there is reason to believe that at least two types of congenital non spherocytic hæmolytic anæmia exist and that of the patients described by Dacie and colleagues (1953) Cases 1 and 4 belonged to one type and Case 5 to a second distinct type The exact position of Cases 2 and 3 in relation to the other patients is uncertain Since the above mentioned paper was written a further patient has been studied (Case 6 below) he appears to be suffering from exactly the same type of disorder as Case 5 referred to above

The two types of disease can be distinguished by a major difference in behaviour on incubation of their blood *in vitro* (see p 110) In addition however there are possible morphological differences for instance the erythrocytes of Case 5 of Dacie and colleagues (1953) and of Case 6 were mostly slightly oval macrocytes (Figs 49 to 51) whereas those of Cases 1 and 4 were (after splenectomy) conspicuously rounded macrocytes (Figs 45 and 48)

Case Report Congenital Non spherocytic Hæmolytic Anæmia Type I (Case 6)

The patient was a boy A M aged 14 years He was born 4 weeks prematurely and was noted to be jaundiced at birth He was said to be pale until 3 months of age At the age of five he was noticed to be jaundiced with darkening of the urine At the age of eleven jaundice reappeared and he was then found to be anæmic His condition has remained unchanged subsequently

Physical Examination When admitted to Hammersmith Hospital for investigation he was found to be an alert boy of average build and development for his age He was visibly jaundiced His spleen was palpable 3 cm below the left costal margin his liver and lymph nodes were normal in size His urine contained urobilin but not bile

Laboratory Findings There were 3 300 000 erythrocytes per c mm with an M C V of 105 c μ the reticulocyte count varied between 8 0 and 11 5% there were 6 000 leucocytes per c mm with 66% neutro phils and 240 000 platelets per c mm Stained blood films showed a tendency to macrocytosis with slight anisocytosis and ovalocytosis and a moderate degree of polychromasia There was no obvious sphero cytosis (Fig 51)

The serum bilirubin concentration was 1 8 to 2 1 mg per 100 ml fæcal urobilinogen 550 mg per day serum albumin 5 1 g per 100 ml serum globulin 1 8 g per 100 ml The Wassermann and Kahn reactions were negative the antiglobulin (Coombs) test was negative and the cold agglutinin titre <4 Osmotic fragility was normal initial lysis

0 45% NaCl M C F 0 40% NaCl complete lysis 0 20% and the increase on incubation at 37 C for 24 hours was normal The rate of auto hæmolysis was normal but this was not diminished to the normal extent by the addition of glucose

Family History No relative is known to have suffered from anæmia or jaundice in particular an elder brother is apparently normal The father s blood was examined and found to be normal That of the mother was however definitely abnormal many of her erythrocytes were slightly oval in shape the M C V was 104 c μ On incubation her blood behaved in exactly the same way as her son s blood i e the rate of autohæmolysis was normal but the effect of glucose on diminishing autohæmolysis was less than normal

Pathogenesis of Congenital Non spherocytic Hæmolytic Anæmia

Very little is known of the nature of the erythrocyte defects in hæmolytic anæmias of the type now being considered or how the defects shorten the life span of the corpuscles *in vivo* It is certain though that the mechanism of hæmolysis differs from that of hereditary spherocytosis in particular a rapid rate of erythrocyte destruction *in vivo* is not dependent upon the presence of a spleen as is shown by the fact that splenectomy has little or no therapeutic value Some light has been thrown on the problem by the recent studies of Selwyn and Dacie (1954) Three of the patients described by Dacie and co workers (1953) and one further patient (Case 6) have been reinvestigated As already mentioned it was found possible to separate the patients into two groups by means of studies *in vitro* Type I comprised Case 5 of Dacie and co workers (1953) and Case 6 (p 108) Type II comprised Cases 1 and 4 of Dacie and co workers

The erythrocytes of both patients of Type I varied only slightly in size some of the cells were macrocytes and many were ovoid in shape On incubation the cell volume and cation changes were similar to those of normal corpuscles as was the increase in osmotic fragility and autohæmolysis However a definite abnormality was demonstrable for when glucose was added to the blood the rate of autohæmolysis was diminished by less than the normal amount The rate of autohæmolysis of blood from the mothers of both these patients was similarly abnormally high in the presence of glucose

The erythrocytes of the patients of Type II behaved quite differently from those of Type I The cells of both these cases were rounded macrocytes few if any being oval During incubation the potassium losses were much greater than normal and the cation and volume changes were unaffected by the addition

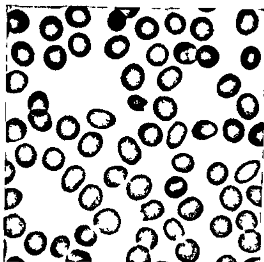


FIG. 31 Photomicrograph of a blood film of a patient suffering from a congenital non spherocytic hæmolytic anæmia (Case 6) × 700

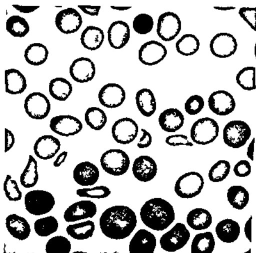


FIG. 32 Photomicrograph of a blood film of a patient suffering from a macrocytic type of congenital hæmolytic anæmia (Case 10 of Dacie *et al* 1953) × 700

of glucose Autohæmolysis was markedly increased in both cases and this was similarly unaffected by glucose Further experiments indicated that the erythrocytes of these patients were unable to utilize glucose at the normal rate—the observed utilization was only 25% and 30% respectively of the calculated amounts

These observations demonstrate that the erythrocytes of the two groups of non spherocytic cases and the erythrocytes of hereditary spherocytosis all behave differently on incubation *in vitro* The essential nature of the corpuscular defects of all three types remains to be determined In the Type I non spherocytic type studies *in vitro* provide no real clue as the behaviour of the cells is normal except for the diminished effect that glucose has on preventing autohæmolysis Nevertheless it is obvious that the defect is one which seriously diminishes the life span of the corpuscles *in vivo* In the Type II non sphero cytic type studies *in vitro* demonstrate a definite and striking abnormality The corpuscles although not originally spherocytic become markedly so on incubation and undergo a striking increase in osmotic fragility and at the same time hæmolyse at a rate that is ten or more times the normal These changes seem to be associated with a metabolic defect—a failure to utilize glucose at the normal rate The nature of the metabolic defect has not yet been defined the rapid onset of spherocytosis *in vitro* suggests that an important consequence of the defect or of an additional defect is a rapid irreversible contraction of the cell membrane

Congenital Non spherocytic Hæmolytic Anæmia Associated with Porphyria

There are a small number of recorded instances of hæmolytic anæmia associated with congenital porphyria (de Marval and Pons 1934 Aldrich Hawkinson Grinstein and Watson 1951 Gray and Neuberger 1952)

Splenectomy was carried out in de Marval and Pons s patient follow ing operation hæmolysis was greatly reduced and the photosensitivity of the skin became less marked The patient of Aldrich and his asso ciates was a little girl aged 4 years Her spleen was also removed Before the operation she was severely anæmic her erythrocytes were slightly macrocytic some were said to be small and spherocytic and curious granulation was noted in the circulating erythrocytes and in the normoblasts in the marrow Following splenectomy her anæmia disappeared and so did the photosensitivity The authors attributed this to a reduction in the synthesis of porphyrins associated with the diminution in erythropoiesis following alleviation of the hæmolytic process Splenectomy was also carried out on Gray and Neuberger s patient in this instance however neither the blood picture nor the photosensitivity was favourably affected

Sato and Takahashi (19?6) described the occurrence of fatal porphyri nuria in a child Terminally the child developed a severe degree of

Morphological and other differences between the erythrocytes in congenital non spherocytic hæmolytic anæmia, Types I and II and hereditary spherocytosis

Disease	Erythrocytes	Osmotic fragility		Autohæmolysis	
		(Before incubation)	(After incubation 4 hrs 37°C)	*Without* added glucose	*With* added glucose
Type I Non spherocytic	Round or oval macrocytes	Normal	Increased but not more than normal Fragility of some cells diminished	Normal	Diminished by less than normal amount
Type II Non spherocytic	Round macrocytes	Normal	Greatly increased	Greatly increased (× 10–20 normal)	*Not* diminished by glucose
Hereditary spherocytosis	Round microspherocytes	Increased	Greatly increased	Increased (× 5–10 normal)	Diminished by normal amount

KAPLAN E and ZUELZER W W (1950b) Erythrocyte survival studies in childhood III Unusual familial hemolytic anemias associated with intrinsic erythrocyte abnormality *J Lab clin Med* 36 524

LIPTON E L GROSSMAN H J and RICHMOND J B (1953) Chronic familial nonspherocytic hemolytic anemia *Pediatrics* 12 384

MARVAL DE L and PONS R (1934) Concomitancia entre porfinuria congenita e ictericia hemolitica Esplenectomia *Arch argent Pediat* 5 220 quoted by Aldrich *et al* (1951)

SATO A and TAKAHASHI H (1926) A new form of congenital hematoporphyrinuria *Amer J Dis Child* 32 325

SELWYN J G and DACIE J V (1954) Autohemolysis and other changes resulting from the incubation in vitro of red cells from patients with congenital hemolytic anemia *Blood* 9 414

THOMPSON W P (1939) Hemolytic jaundice *Bull N Y Acad Med* 15 177

VECCHIO F and TROPEANO L (1947) Su una particulare sindrome anemico-emolitica con ellittocitosi macrocitica in un lattante *Pediatria* 55 240

chlorotic anæmia It seems possible that the anæmia was due to a disturbance in hæmoglobin formation (associated with the excessive formation of porphyrins) rather than due to hæmolysis

Miscellaneous Types of Atypical Congenital Hæmolytic Anæmia

A Macrocytic Type Dacie and co workers (1953) described an unusual macrocytic type of congenital hæmolytic anæmia in a young man aged 19 years Until the age of 15 he had considered himself to be quite well Since then he had been continuously jaundiced and a number of small indolent ulcers had developed on his shins and above his ankles His erythrocytes were unusually macrocytic and varied considerably in size and shape (Fig 52) Their osmotic fragility was normal or slightly diminished No definite evidence of a familial incidence could be established

Splenectomy was carried out but without improvement to his anæmia His jaundice however was slightly lessened After operation his erythrocytes became even more macrocytic (M C D 9 3 μ) An unusual feature in this boy s bone marrow was the presence of quite large numbers of plurinucleated erythroblasts The plurinucleated erythroblasts and the unusual degree of macrocytosis and poikilocyto is in the peripheral blood suggested that the diminished life span of his erythrocytes was secondary to some unusual defect in erythropoiesis

The type of macrocytic anæmia described above is believed to be rare It is possible that the patients described by Fanconi (1939) and Vecchio and Tropeano (1947) were suffering from a somewhat similar disorder Splenectomy was ineffective in Fanconi s patient

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DACIE J V MOLLISON P L RICHARDSON NANCY SELWYN J G and SHAPIRO L (1953) Atypical congenital hæmolytic anæmia *Quart J Med* N S 22 79

FANCONI G (1939) Die verschiedenen Formen der primaren hamolytischen Anamie *Arch Kinderheilk* 117 1

FEINBERG A W and WATSON J (1951) Nonspherocytic chronic hemolytic anemia with basophilic stippling report of a case in a negro *Blood* 6 357

GRAY C H and NEUBERGER A (1952) Effect of splenectomy in a case of congenital porphyria *Lancet* i 851

HADEN R L (1947) A new type of hereditary hemolytic jaundice without spherocytosis *Amer J med Sci* 214 255

HOLLIDAY, T D S (1953) Familial non spherocytic hæmolytic anæmia *J clin Path* 6 219

KAPLAN E and ZUELZER W W (1950a) Familial non spherocytic hemolytic anemia *Blood* 5 811

used in descriptions of the disease It is now realized that both serious and benign forms of the disease are comparatively commonly found chiefly in people of Mediterranean origin On the Continent the disorder has been extensively studied in recent years particularly in Italy and it has now a large literature (see Chini and Valeri 1949 Astaldi Tolentino and Sacchetti 1951)

Cooley Witwer and Lee (1927) at first considered that they were dealing with a familial anæmia of hæmolytic type Later they considered that the anæmia was primarily due to a metabolic disturbance and likened the defective erythropoiesis to attempts to make bricks without straw (Cooley and Lee 1932) As already mentioned it is now realized that both mechanisms are operative and that the hæmolysis is secondary to and probably less important than defective hæmopoiesis Nevertheless Italian authors in particular have referred to patients in whom hæmolysis has seemed to be a dominant feature as suffering from itteri emolitici con aumento della resistenza globulare In Italy this type of anæmia has come to be known as hæmolytic anæmia of the Rietti Greppi Micheli type (see Marmont and Bianchi 1948 Chini and Valeri 1949)

Racial Incidence The great majority of instances of Mediterranean anæmia have been found in people of southern European origin or in their descendants overseas Thus the disease is not uncommon in Italians Sardinians Sicilians and Greeks It has also been observed in Cyprus and Malta and occasionally elsewhere in the Mediterranean littoral It also apparently affects although very much less commonly many different types of non Mediterranean people (Silver 1950 Wintrobe 1951 March Schlyen and Schwartz 1952) Cases have for instance been recorded in Chinese (Foster 1940 DeMarsh 1950) in Germans (Heilmeyer Muller and Schubothe 1951 Pribilla 1951) in Indians (Napier Shorten and Das Gupta 1939 Dhayagude 1944) in Negroes (Schwartz and Mason 1949 Banks and Scott 1953) and in people of pure Thai extraction (Minnick *et al* 1954) It is however not quite clear whether or no the disease in patients of non Mediterranean origin is exactly the same as the type affecting people of Mediterranean stock Probably other rather similar syndromes of different pathogenesis exist (see p 129)

Inheritance It is now generally recognized that Mediterranean anæmia exists in two main grades of severity (Valentine and Neel 1944) thalassæmia major (Cooley s anæmia) a serious disorder which is usually fatal in childhood and thalassæmia

CHAPTER 5

THE CONGENITAL HÆMOLYTIC ANÆMIAS

IV MEDITERRANEAN ANÆMIA AND ALLIED DISORDERS PERNICIOUS ANÆMIA

In this chapter will be described certain congenital anæmias due primarily to defective hæmoglobin synthesis This leads to the formation of erythrocytes very low in hæmoglobin content and usually very variable in size and shape The life span of the most defective of these corpuscles is considerably reduced For this reason in Mediterranean anæmia the most frequently encountered anæmia of this group excessive hæmolysis may be correctly regarded as playing a part in the causation of the patient s anæmia

In pernicious anæmia too although hæmoglobin synthesis is not directly affected the situation is analogous The anæmia is primarily due to dyshæmopoiesis but the abnormal erythrocytes produced as the result of this also have a diminished life span In pernicious anæmia as well as in Mediterranean anæmia there fore a secondary hæmolytic element contributes to the severity of the patient s anæmia The evidence for hæmolysis in pernicious anæmia will be briefly referred to at the end of this chapter

MEDITERRANEAN ANÆMIA

Synonyms Cooley s anæmia (Kato and Downey 1933) erythroblastic anæmia (Cooley and Lee 1932) Mediterranean disease—thalassæmia (Whipple and Bradford 1936) target cell anæmia (Dameshek 1940) familial microcytic anæmia (Strauss Daland and Fox 1941) Rietti Greppi Micheli anæmia (see Marmont and Bianchi 1948) Mediterranean hæmopathic syndrome (Chini and Valeri 1949) hereditary leptocytosis (Committee for Classification of Nomenclature etc 1950)

History The first descriptions of Mediterranean anæmia as *a distinct entity are those of Cooley and Lee (1925)* and Cooley Witwer and Lee (1927) who described a number of children suffering from splenomegaly with anemia and peculiar bone changes Later the eponym Cooley s anæmia was widely

not infrequent As the child grows widening of the cranial bone diploe may lead to enlargement of the skull and often to a mongoloid appearance The spleen may become greatly enlarged

Radiological examination of the child s bones typically reveals thinning of the cortical compact bone and resorption of trabeculæ The outer table of the skull may become extremely thin and the diploe greatly widened Characteristic perpendicular striæ may appear between the inner and outer tables (Cooley Witwer and Lee 1927 Baty Blackfan and Diamond 1932 Caffey 1937 1951) Intractable ulcers of the leg occasionally occur (Estes Farber and Stickney 1948 March Schlyen and Schwartz 1952) and gall stones have been recorded (Currin and Lieberman 1951 Smith and Morgenthau 1951)

Astaldi Tolentino and Sacchetti (1951) referred to three grades of thalassæmia major (1) a severe form causing serious anæmia early in infancy and often resulting in death in the first year (2) a slightly less severe form of the disease usually first becoming manifest in the second half of the first year the child often surviving until school age and (3) a milder form usually diagnosed in the second year of life and compatible with survival until adult life Bone lesions were particularly conspicuous in patients belonging to the second and third groups The literature dealing with the occurrence of thalassæmia major in patients surviving until adult life is reviewed by March Schlyen and Schwartz (1952) who add two more cases of their own

Blood Picture Anæmia is generally severe the erythrocyte count lying as a rule between 1 000 000 and 3 000 000 cells per c mm The erythrocytes vary greatly in size and shape both microcytes and macrocytes being present and many are unusually flattened (Baty Blackfan and Diamond 1932 Bradford and Dye 1936) Small fragments of cells are not infrequently encountered In stained films the appearances are those of extremely severe hæmoglobin deficiency most of the corpuscles staining very palely (Fig 53) Some cells appear as rings of hæmoglobin with little or no staining in the middle other cells present as target cells Normoblasts are almost invariably present the greatest numbers being found in the most severe cases Some of the normoblasts are primitive in others the nucleus is pyknotic and the cytoplasm apparently ripened A moderate degree of polychromasia and punctate basophilia is usually seen The reticulocyte count is usually above normal and may reach 10 per cent or even more Bradford and Dye (1936) recorded the mean corpuscular diameter (M C D) in 8 patients as ranging from 5 8 to 7 4 μ After splenec

minor (target cell anæmia or target oval cell syndrome (Dameshek 1940 1943)) a less serious and not fatal disorder More recently Astaldi Tolentino and Sacchetti (1951) have referred to a third very mild form as thalassæmia minima

It was realized by Angelini (1937) and Caminopetros (1938) that *when one member of a family suffered from severe Mediterranean* anæmia other members of the family commonly suffered from the disease in a minor form Both Angelini and Caminopetros stressed that increased osmotic resistance was a valuable sign of the minor (carrier) state In two of the families studied by Caminopetros both parents were found to be affected and Angelini reported that almost all the available relatives of the 6 families he studied were carriers of the trait These important observations were confirmed and elaborated by Wintrobe (1942) Smith (1942) and Dameshek (1943) in America and by other Italian workers (see Chini and Valeri 1949) and it is now realized that studies on the blood of parents of children affected with Cooley s anæmia will regularly reveal minor but definite hæmatological abnormalities on both sides of the family

It is now generally considered that the severe major form of *the disease represents the homozygous state of a partially dominant* autosomal gene and that the minor and minima forms represent the heterozygous condition (Valentine and Neel 1944 Smith 1948 Chini and Valeri 1949 Astaldi Tolentino and Sacchetti 1951 Ludwin Limantani and Dameshek 1952 Bianco *et al* 1952) A possible but less widely accepted alternative hypothesis is that the severe disease results from the simultaneous presence of two non allelomorphic genes each single gene by itself resulting in only the minor form (Daland and Strauss 1948) Ludwin and co workers (1952) failed to demonstrate any linkage between the gene for Mediterranean anæmia and those for the ABO and Rh blood groups and eye colour

CLINICAL AND HÆMATOLOGICAL FEATURES

Thalassæmia Major (Homozygous State) or Cooley s Anæmia

The disease is usually diagnosed in the first years of life anæmia often becoming marked within a few weeks of birth Pallor is the predominant sign and this is accompanied by swelling of the abdomen due to splenomegaly and to a lesser extent to enlarge ment of the liver Overt jaundice is unusual Purpura and lymph node enlargement do not as a rule occur Bouts of pyrexia are

Data obtained from a child aged 8 years suffering from the disease in its typical form and from the child s parents are given in Table 4

The osmotic fragility of the erythrocytes is characteristically abnormal (Fig 53) The resistance to hæmolysis of the majority of the patient s corpuscles is increased but there may be in addition a small percentage of abnormally fragile cells (Baty Blackfan and Diamond 1932) Hæmolysis is often incomplete in 0 2% saline and sometimes even in 0 1% saline

The changes resulting from the incubation at 37 C of the blood of a patient with severe Mediterranean anæmia were studied by Selwyn (1953) He found (a) that the rate of autohæmolysis was at the upper limit of normal (0 8% at 24 hours and 3 3% at 48 hours) (b) that the cell volume diminished instead of increasing as is normal (c) that the loss of potassium from the corpuscles was greater than normal and (d) that the erythrocyte osmotic fragility was markedly diminished rather than increased as the result of the 24 hours incubation Thus there is some evidence that the Mediterranean anæmia erythrocyte behaves abnormally on incubation as well as being morphologically abnormal

Thalassæmia Minor and Minima (Heterozygous State)

The symptoms produced by the disease in the heterozygous state are far less serious than in thalassæmia major (homozygous state) and most patients are capable of leading moderately active lives (Wintrobe Matthews Pollack and Dobyns 1940 Strauss Daland and Fox 1941 Dameshek 1943) In the mildest cases there may be no complaints attributable to the disease although hæmatological examination reveals definite abnormalities (thalassæmia minima or the microcytemia of Silvestroni and Bianco 1946) In the less fortunate patients the disease results in chronic anæmia of mild to moderate degree and in these cases vague tiredness and dyspnœa on exertion are common complaints (thalassæmia minor) In some patients chronic jaundice of acholuric type is a feature (Rietti Greppi Micheli disease of Italian authors) The spleen is generally palpable in the moderately severely affected patient and ulcers of the leg and gall stones have been observed (Marmont and Bianchi 1948) X ray studies may reveal some degree of osteoporosis and certain physical stigmata such as broadening of the nose and prominence of the cheek bones may also be present

Blood Picture Most patients have a mild to moderate

tomy in two patients the M C D was greater than normal 8 0 to 8 3 μ and 7 5 to 7 7 μ respectively

The leucocyte count is usually raised and may even exceed 25 000 cells per c mm A small percentage of myelocytes is commonly found The platelet count is generally normal

TABLE 4 *The blood counts and other hæmatological data of a child suffering from severe Mediterranean anæmia (thalassæmia major) and of his parents both of whom were carriers of the Mediterranean anæmia trait (thalassæmia minor)*

Patient	Erythrocytes millions per mm	Hæmoglobin g per 100 ml	M C V c.μ	M C H C g	Reticulocytes	Normoblasts per c mm	Serum bilirubin mg per 100 ml	Fœtal hæmoglobin
H M (aged 3)	4 1	6 0	54	23	8 4	8 000	1	1 – 0
Father of H M	6 5	14 1	74	29	2 4	0	—	0
Mother of H M.	5 4	1 7	77	31	2	0	—	0

The plasma bilirubin level is usually slightly raised The serum iron level is high (Cartwright Huguley Ashenbrucker Fay and Wintrobe 1948) and the iron binding protein fully saturated (Smith Sisson Floyd and Siegal 1950)

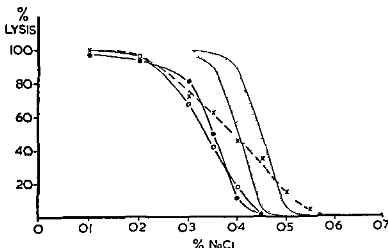


FIG 3 Osmotic fragility curves of the blood of a child suffering from severe Mediterranean anæmia (thalassæmia major) x – – – x and of his parents both of whom were carriers of the Mediterranean anæmia trait (thalassæmia minor) ●———● and o———o

The shaded area represents the normal range

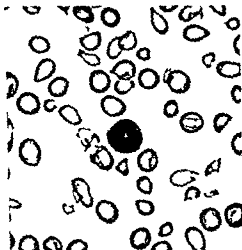


Fig. 34. Photomicrograph of a blood film of a child suffering from severe Mediterranean anæmia (thalassæmia major) × 700

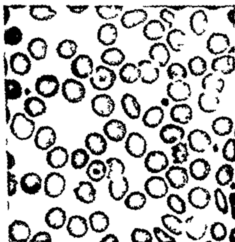


Fig. 35. Photomicrograph of a blood film of a carrier of the Mediterranean anæmia trait (thalassæmia minor) × 700

[To face p. 1..]

anæmia with their hæmoglobin levels not usually reduced below 10 g per 100 ml However in the minima state the hæmoglobin level is usually normal The erythrocyte counts are less reduced often they are within the normal range not infrequently the counts may exceed 6 000 000 cells per c mm (Table 4) The proportion of reticulocytes is generally above normal but seldom exceeds 5% (Smith 1943 Valentine and Neel 1944)

Erythrocyte Morphology Definite abnormalities in erythrocyte morphology are probably always to be found if sought for even in patients without anæmia or where there is erythrocytosis The erythrocytes vary more than normally in size The mean cell volume is usually well below normal (Smith 1943 Valentine and Neel 1944 Heinle and Read 1948 Daland and Strauss 1948) The *mean cell diameter on the other hand is generally* within the normal range (Mooney 1952) the presence of some macrocytes balancing the effect of numbers of microcytes Characteristically the mean cell thickness is considerably reduced (leptocytosis) (Dameshek 1940 Wintrobe *et al* 1940 Smith 1943)

The erythrocytes stain palely with Romanowsky dyes, this is mostly due to their diminished thickness as the hæmoglobin concentration as a rule is only slightly reduced and may be normal (Smith 1943 Valentine and Neel 1944 Daland and Strauss 1948 Heinle and Read 1948) A ring type of staining is characteristic in some cases too target cells are present *(Fig 55) Often many of the erythrocytes are moderately oval in* shape (Dameshek 1940) Punctate basophilia is often conspicuous (Smith 1948 Rietti 1950 Mooney 1952) On the whole the changes are far less severe and the cell morphology more uniform than in the major form of the disease On the other hand the abnormalities are relatively severe in relation to the mildness *of the anæmia that may be present Normoblasts and myelocytes* are not usually present in the peripheral blood

Osmotic Fragility Characteristically the resistance to hypotonic saline is markedly increased and this is found to be present to some extent even in the absence of anæmia (Smith 1943 Valentine and Neel 1944 Mooney 1952) The plasma bilirubin levels are normal or slightly increased As in the major form of the disease the serum iron and copper levels are normal or above normal and the iron binding capacity of the serum may be saturated (Cartwright *et al* 1948 Smith *et al* 1950)

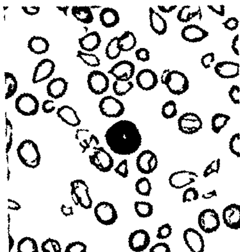


Fig. 54. Photomicrograph of a blood film of a child suffering from severe Mediterranean anæmia (thalassæmia major) × 700

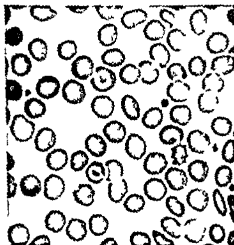


Fig. 55. Photomicrograph of a blood film of a carrier of the Mediterranean anæmia trait (thalassæmia minor) × 700

[To face p. 1[illegible]]

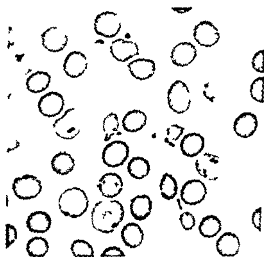


FIG 56 Photomicrograph of a blood film of a boy suffering from a congenital hypochromic anæmia (? distinct from Mediterranean anæmia) After splenectomy and after blood transfusion (see text p 160) × 700

Pathology

Bone Marrow in Thalassæmia The bone marrow is hyperplastic the degree of hyperplasia varying directly with the severity of the anæmia The hyperplasia is the result of active erythropoiesis and in severe cases the erythroid myeloid ratio may exceed unity Erythropoiesis is normoblastic There is a tendency for the developing normoblasts to be smaller than normal (micronormoblastic development) this is mostly due to diminution in the amount of cytoplasm and is most marked in the most ripened cells Detailed measurements are given by Astaldi Tolentino and Sacchetti (1951) In thalassæmia major the percentage of basophilic normoblasts is often unusually high in thalassæmia minor polychromatic and pyknotic normoblasts predominate Astaldi and Tolentino (1952) claimed that in the most serious cases of thalassæmia major there was some delay in the enucleation of the orthochromatic normoblasts Pyknotic normoblasts the cytoplasm of which appears to be completely ripened are in fact not infrequent such cells are rare in normal marrows

In severe cases hæmoglobin may appear to be formed in a patchy fashion in the cytoplasm of the developing normoblasts areas of eosinophilic hæmoglobin being interspersed between remnants of the primitive basophilic cytoplasm This gives rise to an appearance of rather coarse and irregular punctate basophilia

In striking contrast to the finding in simple iron deficiency siderotic granules are present in many of the marrow normoblasts (see p 21)

Other Organs *Spleen* The spleen is usually markedly enlarged Sections show congestion extramedullary hæmopoiesis and a thickened reticulum Deviation from the normal is far less marked in thalassæmia minor than in the major disease The amount of iron present depends upon the number of times if any the patient has received blood transfusions In the absence of a history of transfusions only relatively small amounts of iron are present in the spleen

Liver The iron content of the liver is moderately increased even in the absence of transfusions and the same is apparently true of the iron content of the kidneys heart pancreas and lymph nodes etc (Whipple and Bradford 1936 Astaldi Tolentino and Sacchetti 1951)

Diagnosis of Mediterranean Anæmia

Thalassæmia Major The disease is diagnosed from a consideration of the clinical and hæmatological data and from family studies The rather variable clinical form of the major disease has already been referred to (p 117) In the most severe type affecting infants with many primitive erythroblasts in the peripheral blood confusion with erythræmic myelosis (di Guglielmo s disease) may arise However in the latter disorder myeloblasts will probably be found *in quite large numbers in the* peripheral blood—these are usually absent in Mediterranean anæmia The erythrocytes too will not be conspicuously hypochromic Knowledge of the family history and of the blood picture in relatives will also help in arriving at the correct diagnosis

In the less severe forms occurring in childhood the clinical history and blood picture are generally typical and there should be no real difficulty in diagnosis The same applies to the few patients *who reach adult life*

Thalassæmia major can be distinguished from severe iron deficiency anæmia by the more severe changes in the erythrocytes in the former disease and by study of the serum iron level or iron content of the bone marrow—low in iron deficiency anæmia high in thalassæmia and by the fact that the patient suffering from Mediterranean anæmia fails to benefit from intensive iron therapy

Thalassæmia Minor and Minima Here the separation from iron deficiency anæmia is less easy On clinical grounds the facies of the patient his history the degree of enlargement of the spleen and the possible slight jaundice all point away from iron deficiency as the cause of the anæmia It may be difficult to decide on the blood picture alone hypochromasia anisocytosis and the presence of oval cells and elliptocytes target cells and punctate basophilia all characteristic features of thalassæmia minor may all be found in true iron deficiency anæmia to a greater or lesser degree The same applies to increased erythrocyte osmotic resistance However it is probably true that the numbers of target cells and cells showing punctate basophilia are less likely to be as high and the diminution in osmotic resistance less likely *to be as severe in simple iron deficiency anæmia as in* Mediterranean anæmia Once again knowledge of the serum iron level and bone marrow content of iron the response to iron therapy and the results of family studies are usually decisive in diagnosis

As already mentioned patients with the mildest forms of Mediterranean anæmia may have abnormally high erythrocyte

counts This type of blood picture may be confused with other forms of polycythæmia In the Mediterranean anæmia group the hæmoglobin levels will be found to be normal or subnormal the erythrocytes hypochromic and microcytic and the leucocyte and platelet counts normal Similarly blood containing many conspicuously oval or elliptical erythrocytes may be confused with that of hereditary elliptocytosis In the latter disorder the degree of elliptocytosis is usually more pronounced and regular and the number of cells affected by the change as a rule much greater than in thalassæmia The cells in true elliptocytosis are normochromic not hypochromic and poikilocytosis is much less conspicuous than in Mediterranean anæmia

Treatment of Mediterranean Anæmia

Nothing has yet been found that will produce a sustained favourable effect on erythropoiesis Iron therapy either by mouth or intravenously is useless—indeed the iron binding globulin of the plasma is usually already saturated with iron and the storage organs of the body also contain an excess All the vitamin preparations that have been tried also seem to be useless It is possible but hardly proven that cobalt may be of slight value Berk Burchenal and Castle (1949) reported rather doubtful improvement in one patient and other instances of possible benefit have been referred to by Weissbecker (1951) Muratore (1951) and Heilmeyer Muller and Schubothe (1951) Virdis (1952) on the other hand did not observe any improvement in 6 children given 20 mg of cobaltous chloride orally for 10 to 20 days

Blood Transfusion This has no fundamental effect on the course of the disease However normal blood survives normally in uncomplicated Mediterranean anæmia and for this reason great temporary benefit can be expected to result from transfusion (Hamilton Sheets and DeGowin 1950 Frontali and Stegagno 1951) The use of repeated transfusion will in time lead to marked hæmosiderosis but this in the author s opinion should not be used as an argument against the use of periodic transfusion in cases where without transfusion the degree of anæmia leads to serious symptoms Frumin Waldman and Morris (1952) referred to a child who died at the age of ten having received over 76 litres of blood since birth At post mortem there were all the signs of exogenous hæmochromatosis with early active portal cirrhosis of the liver Fortunately however affected children manage to accommodate themselves remarkably well to hæmoglobin levels

even as low as 5 g per 100 ml if this is so they had best be left untransfused

Splenectomy The spleen has been removed on a number of occasions The general consensus of opinion seems to be that this ordinarily makes little difference to the course of the disease It is likely however that some benefit may follow splenectomy in patients in whom hæmolysis is marked (Govan 1946 Chini and Valeri 1949 Lichtman Watson Feldman Ginsberg and Robinson 1953 Gatto and Lo Iacono 1953 Minnick *et al* 1954)

Lichtman and co workers observed in children suffering from thalassæmia major who had received repeated transfusions that it was quite common for the transfusions to be required at increasingly frequent intervals if the patients hæmoglobin levels were to be maintained They studied by means of the Ashby method the fate of the blood transfused to seven children and found a shortened survival in each case in six of them the half life of the transfused cells was reduced to between five and nine days and in the seventh child it was 35 days This suggested a superadded extracorpuscular mechanism of cell destruction However no abnormal antibodies the presence of which might have explained these findings could be identified

Splenectomy was carried out in five of the patients in four of them the volumes of blood required to be transfused after splenectomy were reduced to 19 21 28 and 36% respectively of the volumes necessary before splenectomy It was concluded that a good case could be made out for removal of the spleen when transfusion studies indicated an abnormal rate of erythrocyte destruction This seemed likely to occur most commonly in patients in whom the spleen was greatly enlarged

Marked erythroblastæmia the presence of many siderocytes an increase in erythrocyte aniso poikilocytosis and an increase in the number of target cells may be expected to follow splenectomy Whipple and Bradford (1936) found that the mean erythrocyte diameter was increased and the erythrocyte thickness decreased after the spleen had been removed



Pathogenesis of Mediterranean Anæmia

There is little doubt but that Mediterranean anæmia is caused by a genetically determined defect of erythrocyte formation As a result abnormally thin mis shapen erythrocytes of low hæmoglobin content are produced which in severe cases at least probably survive for an unusually short time in the circulation Bone marrow hypertrophy follows as a consequence of chronic

anæmia and this in time often leads to the abnormalities of the skull and other bones which are so characteristic of the disease In all except the mildest type of the disease hæmoglobin formation is inadequate despite the hyperplasia of the erythropoietic tissue

The exact nature of the defect of erythrocyte formation has not yet been determined Bone marrow studies show that as the normoblasts grow they develop into unusually small cells which are particularly deficient in cytoplasm The changes are reminiscent of those produced by simple iron deficiency and there seems little doubt that part at least of the fundamental defect of Mediterranean anæmia is a failure of the proper and sufficient synthesis of hæmoglobin in the presence of apparently fully adequate amounts of iron It is possible that the abnormalities of erythrocyte morphology are merely the consequence of this However certain features suggest that a defect in hæmoglobin synthesis may not be the whole extent of the abnormality of erythropoiesis For example the abnormalities in the erythrocytes in thalassæmia major are more severe than are seen in simple iron deficiency anæmia in severe cases too there may be an actual defect in the maturation of normoblasts (Hamilton and Fowler 1951 Astaldi and Tolentino 195-) On the other hand it might be argued that the remarkable changes in erythrocyte morphology and behaviour are all the result of a deficiency of hæmoglobin synthesis and hence of erythrocyte cytoplasm that is more severe than is ever seen in simple iron deficiency It is true too that in the minor and minima varieties the changes in the peripheral blood and in the bone marrow are similar and difficult to distinguish from those produced by simple iron deficiency Even so the degree of punctate basophilia and target cell formation is usually greater than in simple iron deficiency

From the morphological point of view there are therefore some differences between Mediterranean anæmia and simple iron deficiency which do not seem to be entirely explained on quantitative differences in the severity of the impairment of hæmoglobin synthesis There are moreover some other differences between thalassæmia and simple iron deficiency which are probably of pathogenetic significance For instance patients may be encountered in whom jaundice of apparently hæmolytic type is a marked feature These cases appear to be examples of thalassæmia minor in which hæmolysis is unusually pronounced but whether or not the tendency to hæmolysis and jaundice is the result of other genetic influences in addition to that produced by the gene for Mediterranean anæmia is unknown at present

An increased tendency to erythrocyte fragmentation *in vitro* has been mentioned by several writers (Whipple and Bradford 1936) Marmont and Bianchi (1948) in describing three cases of the Rietti Greppi Micheli type reported in detail some observations on this phenomenon The fragmentation was particularly marked in supravital preparations stained with brilliant cresyl blue under these conditions dumb bell erythrocytes appearing as two spheres of hæmoglobin united by a colourless membrane seemed to represent a stage in the fragmentation process These cells could also be found in films of peripheral blood allowed to dry immediately after collection According to Marmont and Bianchi in no other condition except Mediterranean anæmia is evidence for erythrocyte fragmentation so marked It is interesting to note however that they add that in severe simple iron deficiency anæmia the intensity of fragmentation may be almost as great

If the erythrocytes disintegrate *in vivo* in the peripheral blood to a marked extent in Mediterranean anæmia the plasma hæmoglobin concentration would be expected to be abnormally high This has in fact been observed by Crosby and Dameshek (1951) In three patients with severe Mediterranean anæmia the plasma hæmoglobin levels varied from 12 to 60 mg per 100 ml compared with a level of 1 to 4 mg per 100 ml in normal subjects in one moderately severe case the level was normal before splenectomy but 25 mg per 100 ml after splenectomy In 23 patients with the Mediterranean anæmia trait the level was however within the normal range It is interesting to note that in the four patients with raised plasma hæmoglobin levels hæmosiderin could be demonstrated in their urine These studies thus confirm the view that there is a hæmolytic element in Mediterranean anæmia in addition they indicate that erythrocyte disintegration takes place in part at least within the blood stream

The sensitivity of the erythrocytes of patients with Mediterranean anæmia to mechanical trauma *in vitro* is normal or even slightly decreased (Tolentino 1951) It appears therefore likely that fragmentation *in vivo* (and *in vitro*) is an inherent property of the defective erythrocytes

The survival of the erythrocytes of Mediterranean anæmia after transfusion into normal recipients has been studied on several occasions The results indicate that whilst thalassæmia major erythrocytes may have a shortened life span those from patients carrying the Mediterranean anæmia trait probably survive normally

Kaplan and Zuelzer (1950) transfused into normal recipients the blood from three patients with severe or moderately severe Mediterranean anæmia and followed the survival of the transfused erythrocytes by the Ashby method Between 25 and 50% of the transfused cells disappeared from the recipients circulation in 20 to 30 days later however the slope of elimination ran roughly parallel to the expected rate of elimination of normal corpuscles Kaplan and Zuelzer also transfused the erythrocytes from three women carrying the Mediterranean trait into normal recipients The patients were clinically well having erythrocyte counts of between 4 600 000 and 5 300 000 cells per c mm and hæmoglobin levels of between 10 and 11 5 g per 100 ml The survival of their corpuscles in normal recipients was normal The fact that in the severely affected cases some of the cells appeared to be relatively rapidly destroyed while other cells were destroyed at about the normal rate indicates a marked variability within the population of erythrocytes in respect of the defect leading to rapid lysis An examination of a blood film of a severely affected patient certainly shows that there is also a great variability in morphology Kaplan and Zuelzer thought that the most deformed cells were probably eliminated first but concluded that poikilocytosis *per se* was not necessarily associated with rapid elimination

Hamilton Sheets and DeGowin (1950) have also reported on the survival of Mediterranean anæmia trait blood The erythrocytes of a subject with thalassæmia minima survived normally when transfused to a normal recipient but the survival of the corpuscles of a patient with a more severe form of the trait was slightly impaired (elimination complete within 85 days)

Frontali and Stegagno (1951) transfused the blood of two children severely affected with Cooley s anæmia into recipients suffering from mild anæmia not considered to be hæmolytic in origin Using the Ashby method they found that the elimination of the transfused cells was complete in 12 and 19 days respectively

Hæmoglobin in Mediterranean Anæmia

Another difference between Mediterranean anæmia and simple iron deficiency anæmia lies in the fact that a relatively large amount of the hæmoglobin in thalassæmia major is of the fœtal (F) type The first relevant observations were made by Vecchio (1946) and Putignago and Fiore Donati (1948) who showed that the rate of alkali denaturation was slowed Their observations have now been amply confirmed and other points of similarity between fœtal hæmoglobin and Mediterranean anæmia alkali resistant hæmoglobin established (Liquori 1951 Singer Chernoff and Singer 1951 Rich 1952 and Chernoff 1953)

It seems likely that the alkali resistant hæmoglobin in Mediterranean anæmia erythrocytes is in fact identical with fœtal (F) hæmoglobin Liquori (1951) in addition to observing the increased resistance to alkali denaturation also found that the crystal form of the hæmoglobin of one patient resembled that of human fœtal hæmoglobin as described

by Jope and O'Brien (1949) rather than that of human adult hæmoglobin Jope (1949) demonstrated a significant difference in the form and position of the tryptophane notch in the ultra violet absorption spectrum of fœtal hæmoglobin as compared with the normal adult type In thalassæmia major exactly the same difference is discernible (Liquori 1951 Beaven and White 1953)

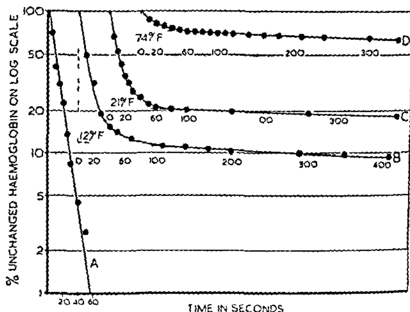


FIG 37 Denaturation rates of hæmoglobin by alkali
A Sickle cell trait No fœtal hæmoglobin present (a normal result)
B Mediterranean anæmia (thalassæmia major) 12% fœtal hæmoglobin present
C Sickle cell anæmia (child aged 1 year) 21% fœtal hæmoglobin present
D Cord blood from normal full term infant 74% fœtal hæmoglobin present (From White and Beaven 1954)

Singer and his co workers (1951) studied 12 patients the blood of all the patients with severe Mediterranean anæmia contained large amounts of fœtal type hæmoglobin as judged by its resistance to alkali On the other hand in those patients with minimal hæmatological signs of the disease the rate of alkali denaturation was normal or only very slightly decreased Rich (1952) studied 11 patients five with thalassæmia major and six with the trait Electrophoretic analysis of trait blood gave a single peak indistinguishable from that of normal hæmoglobin but with thalassæmia major blood two peaks could be resolved one representing the fœtal hæmoglobin and the other normal hæmoglobin The hæmoglobin of two patients with the major type of the disease

who had not been transfused was found to be almost entirely of the fœtal type

Chernoff (1953) showed that there was a very close relationship between the amounts of fœtal hæmoglobin in various types of pathological erythrocytes as estimated by alkali denaturation and the amounts as estimated by an immunological method using a serum prepared against fœtal hæmoglobin obtained from cord blood

Beaven and White (1953) have used a method for studying the rate of alkali denaturation which gives accurate determinations of the amount of resistant hæmoglobin present in a mixture of normal and abnormal hæmoglobin Some results obtained by this method are illustrated in Fig 57

The presence of fœtal hæmoglobin in the erythrocytes in severe Mediterranean anæmia is of great interest but its presence cannot *per se* be held to be responsible in any way for the anæmia After all about 70 to 80% of the hæmoglobin of a healthy newborn infant is composed of this type (Beaven Hoch and Holiday 1951) It is interesting too to note that Singer and his co workers (1951) and Chernoff (1953) have reported the presence of alkali resistant hæmoglobin in increased amounts not only in the erythrocytes of sickle cell anæmia (see p 154) but also in cases of hereditary spherocytosis and in a variety of other anæmias including untreated pernicious anæmia and leukæmia It should be added however that Beaven and White (1953) using several methods of estimation found that fœtal hæmoglobin was practically never present after the first few months of life except in thalassæmia major and sickle cell anæmia

The reason for the persistence of fœtal hæmoglobin in Mediterranean anæmia in sickle cell anæmia and possibly in certain other anæmias is obscure Rich (1952) made the interesting suggestion that the Mediterranean anæmia gene does not in itself cause the formation of an abnormal type of hæmoglobin but rather blocks the formation of the normal adult type and that it is the interference with the synthesis of normal hæmoglobin that leads to the persistence of the fœtal type

SYNDROMES PROBABLY ALLIED TO MEDITERRANEAN ANÆMIA

A small number of families have been reported suffering from types of congenital anæmia similar to but probably not quite identical with Mediterranean anæmia These are referred to tentatively as the *atypical congenital hypochromic anæmias* Subjects of varying nationalities have been affected As in Mediterranean anæmia the essential defect seems to be a failure of the synthesis of hæmoglobin to what extent hæmolysis is important is unknown

The first important contribution was that of Cooley (1945) who under the title A severe type of hereditary anemia with elliptocytosis

reported the incidence of an unusual type of anæmia in two brothers They were members of a family in which for five generations back on the mother s side 19 out of 29 males had suffered from severe anæmia Sixteen of them had died ten in their first year Neither the boys mother nor any other female relative was affected The boys erythrocytes were markedly hypochromic and more than 50% of the cells were oval or elliptical in shape No target cells were present Osmotic fragility studies showed an increased span of resistance there were a few fragile cells but on the whole resistance was increased One boy underwent splenectomy but without definite improvement

Rundles and Falls (1946) described two further American families possibly suffering from the same disorder The first family was of German Scottish and English origin in this family there were two relatively severely affected males and five mildly affected females The second family was of English Dutch and Swiss stock two boys were severely affected and there were six mildly affected females The most obvious blood abnormalities in the mild (carrier condition) were anisocytosis and the presence of some hypochromic elliptocytes and poikilocytes In the severely anæmic males the intensity of the variation in erythrocyte size and shape and staining closely simulated that found in Mediterranean anæmia of a comparable degree of anæmia One of the patients underwent splenectomy without benefit

The author is aware of an as yet unrecorded family of children in London several of whom have suffered from a severe refractory hypo chromic anæmia probably similar to that described by Cooley (1945) and Rundles and Falls (1946) The children s mother has a mild hypochromic anæmia the father s blood is apparently normal The most severely affected child a boy underwent splenectomy No benefit resulted A post splenectomy blood film is illustrated in Fig 56 The patient s hypochromic cells most of which contain a single large siderotic granule contrast strikingly with the transfused normal orthochromic corpuscles

The relationship between the (?) sex linked anæmia of Cooley (1945) and Rundles and Falls (1946) and Mediterranean anæmia is obscure It is not improbable that some of the patients of non Mediterranean origin thought to be suffering from Mediterranean anæmia may have been suffering from the sex linked anæmia At present the only way of differentiating between the two dis orders seems to be by the mode of inheritance The blood pictures do not seem to be sufficiently dissimilar both types are refractory to all forms of medical treatment as well as to splenectomy It is obvious that too few families of the sex linked type have yet been studied for any firm conclusions to be drawn as to whether they are examples of an entity distinct from Mediterranean anæmia

Other (?) Distinct Types of Congenital Hypochromic Anæmia

Stransky and Regala (1946) and Stransky (1951 1953) have described a type of chronic familial hæmolytic anæmia occurring in Filipinos This form of anæmia which the authors considered to be distinct from

Mediterranean anæmia is characterized by a moderate to severe normocytic or slightly microcytic hypochromic anæmia with moderate normoblastæmia considerable reticulocytosis and normal osmotic fragility The disease has been diagnosed at all ages it has a relatively good prognosis and is compatible with a normal span of life Jaundice is usually moderate and splenomegaly marked Crises associated with severe anæmia and jaundice are not infrequent The oldest patient reported by Stransky (1951) was a male of 65 years who was known to have had jaundice and splenomegaly for 45 years Splenectomy does not affect the course of the disease favourably After operation it has been observed that the erythrocytes become macrocytic and that the normoblastæmia increases in intensity

The inheritance of the disease has not been completely worked out possibly it is transmitted as a Mendelian dominant

(f) *Mixed Syndromes*

The possibility of hæmolytic anæmia developing as the consequence of the admixture of two distinct traits for congenital anæmia has already been mentioned (p 101) and will be referred to again in connection with sickle cell anæmia (p 143) According to Quattrin (1950) intermediate types of constitutional hæmolytic jaundice are not uncommon in Italy due he believes to intermarriages between persons carrying the traits for hereditary spherocytosis anæmia of the Rietti Greppi Micheli type and hereditary elliptocytosis respectively The two children described by Debler (1939–40) as suffering from an unusual type of familial hæmolytic anæmia characterized by hypochromia and a markedly increased osmotic fragility may have suffered from a mixed syndrome Both children responded well to splenectomy Further information on this type of case and careful and thorough family studies are badly needed

Pernicious Anæmia

Only a brief reference will be made to pernicious anæmia (P A) as the aspects of the disease other than the evidence for increased hæmolysis are beyond the scope of this book Jaundice is a well known phenomenon in severely anæmic patients and where quantitative studies of the fæcal excretion of urobilinogen have been carried out values greatly exceeding the normal have been found This has been taken as evidence that increased hæmolysis must play a part in the pathogenesis of the disease However the demonstration by means of experiments with ^{15}N labelled glycine that a substantial proportion of the fæcal urobilinogen is derived from sources other than from catabolized hæmoglobin casts some doubt on the validity of this deduction the more so as London and West (1950) showed in a case of pernicious anæmia that as much as 40 per cent of the pigment might be derived from extracorpuscular sources However the total urobilinogen excretion may be many times the normal

(Watson 1931 Barker 1938) and it seems difficult to explain the magnitude of this increase except by postulating increased hæmolysis either affecting adult corpuscles in the blood stream or the nucleated precursors in the bone marrow

The evidence from transfusion experiments seems to be decisive and indicates that the life span of the P A erythrocyte is moderately impaired (Loutit 1946 Singer King and Robin 1948) Loutit transfused the corpuscles of two untreated patients into normal recipients 50% of the cells disappeared by the 10th and 12th days after transfusion respectively Singer King and Robin (1948) transfused to normal recipients the blood of three moderately anæmic patients with hæmoglobin levels between 51 and 56% The survival of these cells was moderately impaired elimination was complete in 27 to 75 days corresponding with 50% survival times of 18 to 30 days approximately The blood of a fourth patient who was in complete remission following liver therapy was also transfused this blood had a normal survival

Further interesting observations have been recently reported by Hamilton DeGowin Sheets Janney and Ellis (1954) who found that when normal erythrocytes were transfused to several patients suffering from pernicious anæmia who had not recently received treatment the corpuscles underwent a slow random destruction which was superimposed on the normal linear rate of decay The random destruction was abolished and a normal survival observed if the patient had received adequate therapy with vitamin B_1 *before* the transfusion was given but not if the vitamin B_{12} was administered afterwards The mechanism or site of the extracorpuscular hæmolytic mechanism was not determined

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McCarty 1930) The sickle cell trait was referred to as sicklemia by Cooley and Lee (1926)

Racial Characteristics The sickle cell phenomenon and sickle cell anæmia are almost entirely confined to the blood of negroes Mason (1938) however accepted as authentic reports of sickling in several white families without any reasonable suspicion of admixture with negro blood Wintrobe (1951) listed 13 instances mostly in Greeks Italians and Sicilians but added that ancestral negro blood can be suspected Margolies (1951) who referred to 30 cases and Plachta and Speer (1952) similarly concluded that ancestral admixture with negro blood was the most likely explanation Recently however the sickle-cell trait has been found to occur relatively frequently in certain localities in Greece (Choremis *et al* 1951) and also in certain primitive hill tribes (Veddoids) in India (Lehmann and Cutbush 1952) Whether the gene responsible for the sickle cell trait has arisen independently in the three ethnic groups or spread to each group from a common ancestor has not yet been settled (Neel 1953) Lehmann (1953) suggested that the sickle cell trait unlike the rhesus gene combination cDe is not an essentially negroid feature he considered that the trait probably entered the African continent from the north east A recent observation of great interest is the apparent association of the sickling trait with resistance to infection by the parasite of subtertian malaria (Allison, 1954)

Inheritance The possibility that the sickle cell phenomenon might be inherited was first hinted at by Emmel (1917) who observed that the blood of the father of a patient suffering from sickle cell anæmia sickled *in vitro* Later it was realized that sickling occurred in two distinct conditions in a peculiar type of anæmia—sickle-cell anæmia and as a symptomless trait—the sickle cell trait (Cooley and Lee 1926 Diggs Ahmann and Bibb 1933–34 Sherman 1940) The distinction between the two forms of disease and other more recent subdivisions were considered by Neel (1952) The presence or absence of the sickling phenomenon appears to be determined by a single gene A child who receives this gene from one parent and a normal gene from the other develops the *sickle cell trait* (heterozygous state) but does not become anæmic On the other hand a child who receives genes for sickling from both parents develops *sickle cell anæmia* (homozygous state) So far no satisfactory evidence has yet been produced for any linkage between the genes responsible for the sickling phenomenon and those responsible for the blood groups

CHAPTER 6

THE CONGENITAL HÆMOLYTIC ANÆMIAS

V SICKLE CELL DISEASE AND ALLIED SYNDROMES

History In 1910 Herrick published an article entitled Peculiar elongated and sickle shaped red blood corpuscles in a case of severe anemia in which are described many of the more characteristic hæmatological and clinical findings of what is now referred to as sickle cell anæmia Although sickle cells were well illustrated by Herrick the development of sickling *in vitro* was not described until 1915 when Emmel studying the blood of a patient whose clinical history was reported by Cook and Meyer (1915) noticed that long sharp projections formed from the erythrocytes when sealed preparations of blood were allowed to stand undisturbed at room temperature for several days In 1917 Emmel published a full description of his observations on the development of sickled forms he also reported that identical changes took place when the blood of the patient s father was similarly cultured *in vitro* Mason (1922) introduced the term *sickle cell anæmia* and suggested that the disease might be confined to the negro race Huck (1923) showed that the sickling phenomenon was unquestionably inherited and suggested that the mode of inheritance was that of a Mendelian dominant

Subsequent discoveries of great significance include those of Hahn and Gillespie (1927) who showed that sickling developed as the result of a fall in the partial pressure of oxygen and the more recent work of Pauling Itano Singer and Wells (1949) who demonstrated that the sickling phenomenon was associated with the presence of an abnormal form of hæmoglobin The work of Pauling and his collaborators has been the starting point of much recent work of major importance In particular two further types of abnormal hæmoglobin in addition to sickle cell hæmoglobin have been discovered (see later)

Sickle cell disease has by now a very large literature Margolies (1951) in a comprehensive review listed 344 references He gives a good account of the early history of the disease

Synonyms Sickle cell anæmia (Mason 1922) drepanocytic anæmia (Hahn 1928) meniscocytic anæmia (Graham and

e g weakness fatigability and dyspnœa As a rule their illness runs a fairly stable course From time to time however exacerbations take place and at these times it is usual for the patients to complain of aching pains in the joints or elsewhere in the limbs and sometimes of abdominal pain and nausea These crises are often associated with pyrexia

Physical examination reveals pallor of the mucous membranes and typically a greenish yellow colour of the conjunctivæ The spleen is usually palpable in children but this is by no means invariable it is not usually palpable in the adult (see p 143) The liver is frequently palpable particularly in children (Margolies 1951 Green Conley and Berthrong 1953) According to Green and co workers there is often clinical evidence of hepatic dysfunction Gallstones are found in about one third of the patients (Weens 1945 Green *et al* 1953) Cardiac enlargement chiefly of the right side of the heart is common and is often more severe than is usually found in chronic anæmia (Klinefelter 1942) Another remarkable but quite common sign is the presence of chronic ulceration of the leg similar to that found in hereditary spherocytosis the ulcers are usually bilateral and lie superficial to or just above the internal or external malleoli Not infrequently neurological complications develop these are probably the result of multiple thromboses

Radiological examination reveals as a rule many interesting abnormalities Excluding evidence of cardiac enlargement which is almost invariable the main changes are found in the bones occasionally areas of calcification in the spleen can be seen (Caffey 1937 Ehrenpreis and Schwinger 1952) Irregularities or abnormalities in the pattern of bony trabeculæ and thickening of the diploe of the skull are characteristic findings (Caffey 1937) Detailed descriptions of these and other clinical features are given by Grover (1947) Margolies (1951) and by Wintrobe (1951)

Sickle cell anæmia has been most commonly reported from the United States of America In Africa despite the fact that the incidence of the trait is high in some areas sickle cell anæmia has been rarely diagnosed It is however not unknown (Foy and Kondi 1952 Edington 1953) It has also recently been recorded in Macedonia (Veras Démétriadès and Manios 1953) and in Upper Assam (Dunlop and Mozumder 1952)

The Blood Picture in Sickle cell Anæmia

Erythrocytes Anæmia is moderate or severe the erythrocyte count is usually between 2 000 000 and 3 500 000 cells per c mm

or other easily recognized inherited characters (Neel Schull and Shapiro 1952)

Occasionally sickle cell anæmia may be found in a child although the erythrocytes of only one of his parents sickle *in vitro* Neel (1952) suggested that the most likely explanation was that the normal parents had contributed other genes which in combination with a single sickle cell gene produced overt sickle cell anæmia Three combinations capable of doing this are now known (Itano 1953a) (1) the non sickling parent contributes the gene for Mediterranean anæmia (thalassæmia) in which case sickle cell anæmia develops in a child heterozygous for the sickle cell and thalassæmia traits (microdrepanocytic disease) (2) the non sickling parent contributes a gene for hæmoglobin C[1] (III) (Itano and Neel 1950 Kaplan Zuelzer and Neel 1951) in which case sickle cell anæmia usually of a mild type occurs in a child heterozygous for both the sickle cell and hæmoglobin C traits and (3) the non sickling parent contributes a gene for hæmoglobin D[1] (Itano 1951) Other abnormal genes acting in a similar way perhaps await discovery The clinical significance and laboratory findings associated with the presence of the sickle cell gene alone or in combination with the genes for thalassæmia or hæmoglobins C or D is dealt with below

CLINICAL FEATURES

(1) Sickle cell Trait (Sicklemia) *heterozygous state one gene for sickle cell hæmoglobin (hæmoglobin S)[1] and one gene for normal hæmoglobin (hæmoglobin A)[1]*

The presence of a single sickle cell gene in combination with a gene for normal hæmoglobin does not lead to anæmia or any other symptoms and stained blood films appear normal It has been found too that the erythrocytes of healthy carriers of the sickle cell trait survive for a normal length of time when transfused to healthy recipients (Singer Robin King and Jefferson 1948 Callender Nickel Moore and Powell 1949)

(2) Sickle cell Anæmia (*homozygous state two genes for sickle cell hæmoglobin*)

The disease is usually diagnosed for the first time in childhood The patients complain of the usual symptoms of chronic anæmia

[1] The nomenclature is that recommended by the Hematology Study Section of the Division of Research Grants of the National Institutes of Health of the United States (*Blood* 8 386 1953)

In fatal cases the usual signs of the effect of chronic anæmia are found in addition to hæmosiderosis the degree of the latter depending largely on the number if any of blood transfusions the patient had had Occasionally ischæmic infarcts are found (Kimmelstiel 1948) and in patients with marked cor pulmonale occlusion of the smaller arteries of the lungs may be observed (Yater and Hansmann 1936) The lesions in the nervous system also appear to be due to intravascular accumulations of sickled cells or to thromboses

The liver is frequently the site of major pathological changes Green Conley and Berthrong (1953) reported on 21 autopsies unequivocal cirrhosis was found in four patients and in many of the others there were active or healed areas of necrosis The necroses were thought to be due to vascular obstruction brought about by impacted masses of sickled cells or by Kupffer cells swollen with phagocytosed erythrocytes

The spleen is enlarged in the early stages of the disease the pulp will then be found to be engorged with sickled erythrocytes Later infarctions and general shrinkage and fibrosis of the organ take place and in the last stages of the disease it may be much smaller than in health and weigh only a few grams (see Margolies 1951)

Aplastic Crises in Sickle cell Anæmia

In 1950 Singer Motulsky and Wile described two children suffering from sickle cell anæmia in whom there was an abrupt increase in the severity of their anæmia This was found to be associated with reticulocytopenia and temporary cessation of erythropoiesis in the bone marrow In both instances the crisis seems to have been precipitated by infections The course of events appears to have been the same as in the aplastic crisis of hereditary spherocytosis (see p 68) More recently Chernoff and Josephson (1951) reported four more instances of aplastic crisis in sickle cell anæmia in three patients the initiating cause seemed to be an upper respiratory tract infection and in one patient infection with *Salm choleræ suis*

Sickle cell Thalassæmia Disease or Microdrepanocytic Disease (*one gene for sickle cell hæmoglobin and one gene for thalassæmia*)

This form of sickle cell anæmia was described by Silvestroni and Bianco (1946 1952) as la malattia micro drepanocitica Subsequently several American families of Italian Sicilian or

but it may fall as low as 1 000 000 per c mm The mean corpuscular volume is generally normal occasionally in the most anæmic cases it is above normal The hæmoglobin concentration is also usually normal (Diggs and Bibb 1939) In stained smears anisocytosis is moderate in extent As a rule a few conspicuously elongated cells with sharp or rounded ends are present some may be oat shaped or sickle shaped Sometimes target cells are present Polychromasia is often marked the cells staining diffusely basophilic being usually round in contour Normoblasts are not infrequently present in small numbers The erythrocyte osmotic fragility is usually moderately diminished Moderate numbers of siderocytes may be found in peripheral blood films even before splenectomy (Kaplan Zuelzer and Neel 1953)

Leucocytes The leucocyte count may be raised to 20 000 cells per c mm or more when hæmolysis is most active The leucocytosis is chiefly due to an increase in the polymorphonuclear neutrophils a few myelocytes may also be present

Platelets The platelet count is usually normal

Plasma Bilirubin The plasma bilirubin level is usually moderately increased as a rule to between 1 and 2 mg per 100 ml

Plasma Proteins According to Fenichel Watson and Eirich (1950) abnormalities in the plasma protein pattern are frequently found Thirteen out of 15 patients with sickle cell anæmia had decreased concentrations of albumin twelve had elevated γ globulin concentrations and three raised concentrations of β globulin The plasma fibrinogen level was high in eight out of ten of these patients Fenichel and co workers suggested that these changes might be non specific reactions secondary to tissue breakdown and that this occurred particularly in the liver as the result of vascular obstruction due to sickling (see *Pathogenesis* p 154)

Pathology

Bone marrow As in other chronic hæmolytic anæmias the erythropoietic tissue is hyperplastic and the erythroid myeloid ratio may be reversed Fat cells tend to disappear The normoblasts in the marrow are morphologically normal However sickled adult erythrocytes may be visible in smears and may often be present in greater numbers than in the peripheral blood The actual volume of red marrow is considerably increased and this leads to widening of the marrow cavities of the bones and changes such as thickening of the diploe of the skull which are visible radiologically

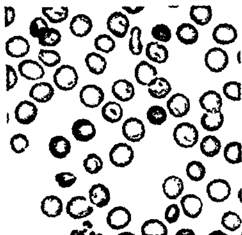


Fig. 8. Photomicrograph of a blood film of a patient suffering from sickle-cell haemoglobin—haemoglobin C disease × 700

Greek origin were found to be suffering from the same syndrome (Powell Rodarte and Neel 1950 Banks Scott and Simmons 1952 Wasserman Phelps and Hertzog 1952 Sturgeon Itano and Valentine 1953 Neel Itano and Lawrence 1953) According to Silvestroni and Bianco (1952) microdrepanocytic disease can be distinguished from true sickle cell disease by hæmatological characteristics as well as by genetical studies

Clinically the disease is usually a fatal one although many patients reach adult life As in true sickle cell anæmia the patient presents with chronic anæmia moderate jaundice hepato splenomegaly chronic ulcerations of the leg recurrent bouts of fever osteo articular pains and sometimes with crises of severe abdominal pain Hæmatologically microdrepanocytic disease is characterized by a severe hypochromic anæmia markedly decreased erythrocyte fragility microcytosis and striking aniso poikilocytosis with many oval cells and target cells Sickle cells are not as a rule visible in freshly made blood films but sickling can be induced *in vitro* The optical properties of the sickled corpuscles are the same as those in the sickle cell trait (Ascenzi and Silvestroni 1953)

Sickle cell Hæmoglobin—Hæmoglobin C Disease *(one gene for sickle cell hæmoglobin and one gene for hæmoglobin C)*

The existence of an unusual type of hæmoglobin (hæmoglobin C) in the blood of certain American negroes was reported by Itano and Neel (1950) The clinical syndrome associated with its presence was first described by Kaplan Zuelzer and Neel (1951) and more recently studies have been reported by Smith and Conley (1953) Neel Kaplan and Zuelzer (1953) and Kaplan Zuelzer and Neel (1953)

The combination of a gene for sickle cell (S) hæmoglobin with a gene for hæmoglobin C results in a hæmolytic syndrome with splenomegaly resembling sickle cell anæmia The disease is however milder and follows a relatively more benign course than typical sickle cell anæmia The splenomegaly persists until adult life (Smith and Conley 1953) Although sickle cells can seldom be found in dried blood films wet preparations of blood sickle in the same sort of way as in the sickle cell trait and micro drepanocytic disease In stained films the most abnormal feature is the presence of many target cells (Fig 58) according to Kaplan Zuelzer and Neel (1953) from 40 to 80% of the erythrocytes may be of this type The anæmia is hypochromic or normochromic and usually slightly microcytic and there is relatively little aniso

poikilocytosis The reticulocyte count is only slightly or moderately raised and normoblasts are rare in films of peripheral blood The erythrocyte osmotic fragility is diminished to about the same extent as in true sickle cell anæmia Small numbers of siderocytes may be present The plasma bilirubin level is normal or slightly increased and the fæcal urobilinogen excretion moderately increased (Kaplan Zuelzer and Neel 1953)

Hæmoglobin C Trait The presence of a single gene for hæmoglobin C combined with a gene for normal hæmoglobin results in well-defined hæmatological abnormalities but no anæmia Smith and Conley (1953) in a study of 500 negro patients found the incidence of the hæmoglobin C trait to be 2% compared with that of the sickle cell trait which was 8 1%

According to Kaplan Zuelzer and Neel (1953) and Smith and Conley (1953) the chief hæmatological feature of the hæmoglobin C trait is the presence of unusual numbers of target cells in the blood without significant degrees of microcytosis hypochromasia or aniso poikilocytosis Occasionally the film may be indistinguishable from normal (Smith and Conley 1953) No sickling occurs *in vitro* In 13 subjects carrying the hæmoglobin C trait the numbers of target cells varied from 3 to 83% (Kaplan Zuelzer and Neel 1953) in four out of seven subjects the erythrocyte osmotic resistance was increased significantly their plasma bilirubin levels were normal Transfusion studies indicated that the life span of erythrocytes carrying the hæmoglobin C trait was probably normal or almost normal (Kaplan Zuelzer and Neel 1953)

The nature and distinguishing characteristics of hæmoglobin C are considered on p 152

Homozygous Hæmoglobin C Recently several patients have been described whose hæmoglobin has been shown by physico chemical studies to consist entirely or almost entirely of hæmoglobin C

As is referred to in a previous paragraph Smith and Conley (1953) found that the incidence of the hæmoglobin C trait in American negroes was approximately 2% It was to be expected therefore that sooner or later subjects inheriting the trait from both parents would be discovered Several examples of hæmoglobin C in the homozygous state have in fact recently been reported (Spaet Alway and Ward 1953 Levin Schneider Cudd and Johnson 1953 Ranney Larson and McCormack 1953) The patients so far described have had few symptoms but seem nevertheless to be subject to a mild hæmolytic process they

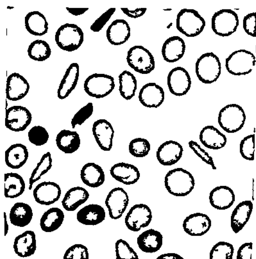


Fig. 59 Photomicrograph of a blood film of a patient suffering from sickle cell hæmoglobin—hæmoglobin D disease (Case 7)

TABLE 5 *Erythrocyte counts and other hæmatological data of a patient R M (Case 7) suffering from a type of sickle cell anæmia and of her parents and two brothers* (S = *sickle cell hæmoglobin* D = *hæmoglobin* D A = *normal adult hæmoglobin*)

Subject	Erythrocytes millions per c mm.	Hæmoglobin g per 100 ml	M C V c.μ	M C H C.	Reticulocytes	Bilirubin g per 100 ml	Type of hæmoglobin	Erythrocyte morphology
R M (patient Case 7)	2 6	8 4	100	3[illegible]	0 0	[illegible] 1	S + D	Some oat shaped and sickled cells much anisocytosis a few target cells and spherocytes
Mr M (father)	4 2	1[illegible] 9	96	31	1 3	0 [illegible]	S + A	Normal
Mrs M (mother)	5 3	16 9	93	3[illegible]	2 8	0 5	D + A	Normal
G M (brother)	5 1	13 8	[illegible]0	34	1 0	—	D + A	Normal
B M (brother)	5 1	16 4	96	33	1 2	—	A	Normal

have been mildly anæmic and have had normal or slightly raised reticulocyte counts Their erythrocytes were normochromic and normocytic and did not sickle but many target cells were to be seen in peripheral blood films Bone marrow aspiration revealed a moderate degree of normoblastic hyperplasia

Sickle cell Hæmoglobin—Hæmoglobin D Disease *(one gene for sickle cell hæmoglobin and one gene for hæmoglobin D)*

Another type of abnormal hæmoglobin (hæmoglobin D) was found by Itano (1951) in several members of a family in which sickle cell anæmia had occurred The distinguishing characteristics and nature of this variety of hæmoglobin are considered on p 153

The clinical syndrome and hæmatological findings of this type of sickle cell disease await definition A probable example of this apparently rare combination is nevertheless described below

Case Report Sickle cell Hæmoglobin—Hæmoglobin D Disease

Case 7 The patient (R M) was a white girl aged 9 years She had been admitted into hospital on two previous occasions two years previously with an unexplained pyrexia She was known to be moderately anæmic but the true nature of her anæmia was not suspected at that time She was referred by Dr S D V Weller to the author for further investigation

Physical Examination The patient was seen to be rather small for her age and to be pale and very slightly jaundiced The lymph nodes in the neck and axillæ were slightly enlarged but neither the liver nor the spleen was palpable There were no other significant physical signs

Neither her colour nor the physical features of the patient suggested a negro ancestry However this was in fact probable Her father was born in Jamaica as he believed of Irish French English and Scottish ancestors Although Caucasian in complexion some of his facial features were slightly negroid The patient s mother was born in England She had Caucasian features some of her remote ancestors were of Austrian and Spanish origin The patient had two brothers the elder had slightly negroid features like the father the features of the younger were Caucasian

Laboratory Findings The patient was moderately anæmic the erythrocyte count was 2 000 000 cells per c mm hæmoglobin 8 4 g per 100 ml M C V 100 c μ M C H C 32% and reticulocytes 9 0% There were 11 000 leucocytes per c mm and 330 000 platelets per c mm A stained peripheral blood film presented a remarkable appearance (Fig 59) there was a marked degree of anisocytosis both macrocytes and microcytes being present and a conspicuous feature was the relatively large number of oat and sickle shaped forms The erythrocytes stained with a variable intensity but most of them were hypochromic a small number were target cells Polychromasia was

cell anæmia than it is in the *sickle cell trait* (Diggs Ahmann and Bibb 1933-34 Neel 1951) The changes certainly occur more rapidly in the anæmia than in the trait

Although sickling occurs at birth it takes place less readily in the blood of newborn infants than in later life (Watson Stahman and Bilello 1948 Scott Crawford and Jenkins 1948) For instance Watson and co workers showed in a series of newborn negro infants that from 0 5 to 29 5% of their erythrocytes sickled compared with 84 to 100% sickling in the blood of the infants mothers One infant s blood was studied at frequent intervals it was found that the proportion of cells that would sickle increased from 0% at birth to 90% at four months It seems possible that the large amount of fœtal hæmoglobin present at birth protects the cells in some way from the effects of a reduced oxygen tension

There are differences too in the ease with which erythrocytes of different ages sickle Watson (1948) found that whereas most reticulocytes sickled as readily as adult corpuscles the most immature ones sickled more slowly whilst mature normoblasts sickled more slowly still Watson also noticed that the sickled cells seen in stained smears of air dried films of peripheral blood were almost invariably adult non reticulated corpuscles

Hahn and Gillespie (1927) seem to have been the first to have demonstrated that sickling *in vitro* depended on a reduction in the partial pressure of oxygen Subsequently a number of techniques were evolved for bringing about de oxygenation of the blood more quickly than it occurs in sealed preparations Sodium bisulphite and vitamin C (Daland and Castle 1948) buffered isotonic sodium dithionite at pH 6 8 (Itano and Pauling 1949 Williams and Mackey 1949) and cultures of *Bacillus subtilis* (Singer and Robin 1948) have been added to blood to bring this about (see also Chapter 18)

Sickle cell (S) Hæmoglobin and other Abnormal Hæmoglobins (Hæmoglobins C and D)

As referred to earlier recent studies have demonstrated that an abnormal form of hæmoglobin is present in the erythrocytes in sickle cell anæmia and in the sickle cell trait (Pauling Itano Singer and Wells 1949 Pauling *et al* 1950) This discovery initiated investigations which have gone a long way to explain the phenomenon of sickling Pauling and his co workers found that the electrophoretic mobilities of normal and sickle cell hæmoglobins differed significantly in addition they provided

conspicuous and a very few normoblasts could be found The osmotic fragility of the erythrocytes was generally considerably diminished although there was a small percentage of fragile cells undergoing lysis in 0 55% NaCl (Fig 17) The plasma bilirubin was 2 1 mg per 100 ml serum albumin 4 3 g per 100 ml and serum globulin 3 1 g per 100 ml

The patient s blood underwent rapid sickling when sealed preparations were incubated or when reducing agents were added

Family Studies Blood samples of all the other members of the family who were available were examined The father s blood sickled readily *in vitro* though not so rapidly as the blood of the patient The blood of the patient s mother and that of two brothers did not undergo sickling None of the patient s relatives who were examined was anæmic nor did examination of stained blood films reveal any definite abnormalities However the osmotic resistance of the father s erythrocytes was slightly diminished and the mother s reticulocyte count was slightly above the normal level The hæmatological data on the family are summarized in Table 5

Electrophoretic and solubility studies have been carried out on the hæmoglobins of the members of this family by Dr J C White Briefly his results are as follows the patient s hæmoglobin has the electrophoretic mobility of sickle cell hæmoglobin—it is probably a mixture of S hæmoglobin and hæmoglobin D the father s hæmoglobin is a mixture of S hæmoglobin and normal hæmoglobin the mother s hæmoglobin is a mixture of normal hæmoglobin and hæmoglobin D The hæmoglobin of one of the patient s brothers is normal that of a second brother is a mixture of normal hæmoglobin and hæmoglobin D (Fig 61 (2))

The Sickling Phenomenon

Sickling of erythrocytes is now known to be due to the presence within them of an abnormal type of hæmoglobin (hæmoglobin S) As will be discussed later this type of hæmoglobin is far less soluble than normal hæmoglobin particularly in the reduced state and it seems that the alteration in the shape of the erythrocytes is the direct consequence of changes leading to crystallization of the abnormal hæmoglobin within the cells under conditions of reduced oxygen tension and diminished pH

Sickling was first demonstrated by allowing a fluid preparation of blood to remain sealed beneath a coverslip Under these circumstances gradual deoxygenation takes place and the erythrocytes undergo a progressive distortion in a matter of minutes or hours Eventually in sickle cell anæmia all the cells become changed to crescentic forms some with associated spines or filaments Details of the sequence of changes were well described by Ponder (1945) In other cases the cells become less obviously sickle shaped and may assume instead a holly leaf appearance with multiple spines There is reason to believe that the formation of markedly distorted filamentous sickled forms is greater in *sickle*

still not quite clear Pauling and his co workers (1949) suggested that under conditions of reduced oxygen tension the molecules of sickle cell hæmoglobin underwent a partial alignment within the cells and that elongation of the cells in one axis and distortion followed from this Harris (1950) suggested that linkage of individual molecules led to the formation of long tactoids

Perutz and Mitchison (1950) made some observations of great importance They showed that reduced sickle cell hæmoglobin was far less soluble than normal hæmoglobin Whereas the solubility of reduced normal hæmoglobin was about one half of that of normal oxyhæmoglobin the solubility of reduced sickle cell hæmoglobin was no more than one hundredth of that of sickle cell oxyhæmoglobin They suggested that the sickle cell shape and birefringence of the distorted cells resulted from crystallization of the hæmoglobin within the cell membranes

Further studies of the differences between normal and sickle cell hæmoglobin have been carried out by Perutz Liquori and Eirich (1951) Using a slightly different technique they found that the solubility of reduced sickle cell hæmoglobin was about one tenth of that of reduced normal hæmoglobin As a result of other experiments they concluded that the solubility of reduced sickle cell hæmoglobin was only one seventh of that required to keep the hæmoglobin in solution in the cell Perutz Liquori and Eirich (1951) also found that whereas at least two crystal forms were common to both normal and sickle cell hæmoglobin sickle cell oxyhæmoglobin might crystallize in a third form not produced by normal hæmoglobin

The differences in solubility between sickle cell hæmoglobin and normal adult hæmoglobin provide one method for the distinction between the sickle cell trait and sickle cell anæmia as the observed solubility seems to be a direct reflection of the proportion of sickle cell hæmoglobin present (Fig 60) Itano (1953) found that the solubility of the hæmoglobin mixtures in the different types of sickle cell disease could be ranged in the following order sickle cell trait—sickle cell hæmoglobin C disease—sickle cell thalassæmia—sickle cell anæmia the solubility in sickle cell trait was the highest and that in sickle cell anæmia the lowest

Singer and Singer (1953) in studies in which they measured the minimum concentrations of sickle cell hæmoglobin which would undergo gelling when deoxygenated observed that the gelling point was modified by the presence of other types of hæmoglobin In sickle cell trait for instance the presence of normal adult hæmoglobin diminished the minimal amount of S hæmo

evidence that the difference lay in the globin part of the molecule and not in the hæm

Schroeder Kay and Wells (1950) analysed quantitatively normal adult hæmoglobin and sickle cell hæmoglobin with particular reference to the content of amino acids Very small differences were found but it was thought that they might be sufficient to affect the coiling of the polypeptide chains and to modify indirectly the electrophoretic behaviour of the hæmoglobin

Pauling and co workers (1950) and Wells and Itano (1951) reported that in the carrier of the sickle cell trait (with one gene for sickle cell (S) hæmoglobin) the proportion of S hæmoglobin varied from 25 to 45% in patients with sickle cell anæmia (with two genes for S hæmoglobin) on the other hand the proportion of abnormal hæmoglobin was as high as 80 to 100% (Pauling *et al* 1949 Wells and Itano 1951) Neel Wells and Itano (1951) further showed that in some families carrying the sickle cell gene significantly smaller amounts of abnormal hæmoglobin were developed than in others Neel (1952) explained this by suggesting that other genes (not necessarily those for abnormal hæmoglobins) might influence significantly the amount of abnormal hæmoglobin formed as the result of the presence of the gene for S hæmoglobin

The clinical augmentation of the effect of a single gene for S hæmoglobin which results from the simultaneous presence of a gene for hæmoglobin C (or D) or thalassæmia is associated with the formation of an increased proportion of sickle cell hæmoglobin (Neel Itano and Lawrence, 1953) In two children suffering from sickle cell thalassæmia disease the proportion of S hæmoglobin for instance was found to lie between 61 and 84% a concentration higher than in sickle cell trait but lower than in most instances of true sickle cell anæmia

The presence of a gene for a second abnormal type of hæmoglobin or the gene for thalassæmia is the usual explanation for the finding of overt sickle cell anæmia in a child when the blood of only one of his parents sickles An example of this association has been recorded on p 146 However the presence of unusually small amounts of sickle cell hæmoglobin may be the explanation in rare instances Singer and Fisher (1953a) have reported a possible case in which the blood of the mother of two affected children was found to contain as little as 5% of sickle cell hæmoglobin this was just detectable by electrophoresis but was insufficient to cause sickling in the *in vitro* test

The exact way in which sickle cell hæmoglobin in the reduced state causes such a remarkable distortion of the erythrocytes is

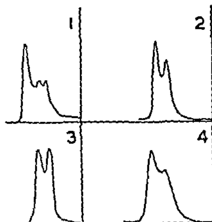


FIG 61 Human hæmoglobin resolved by paper electrophoresis and recorded by scanning with the Laurence densitometer
1 Normal hæmoglobin mixed with hæmoglobin from a case of sickle cell hæmoglobin—hæmoglobin C disease Normal hæmoglobin (large peak) sickle hæmoglobin (left hand small peak) C hæmoglobin (right hand small peak)
2 Hæmoglobin D trait Normal hæmoglobin (larger peak) D hæmoglobin (smaller peak)
3 Sickle-cell hæmoglobin—hæmoglobin C disease Sickle hæmoglobin (left hand peak) C hæmoglobin (right hand peak)
4 Sickle-cell trait Normal hæmoglobin (large peak) sickle hæmoglobin (smaller peak) (From White and Beaven 1954)

indistinguishable from hæmoglobin S by electrophoresis has apparently a normal solubility

Representative electrophoretic patterns with different hæmoglobin combinations are illustrated in Fig 61 The technique of filter paper electrophoresis as applied to the differentiation of the hæmoglobins is described by Smith and Conley (1953) and Larson and Ranney (1953) (see also p 504)

Fœtal (F) Hæmoglobin in Sickle cell Disease Bianco (1948) and Singer and his associates (Singer Chernoff and Singer 1951 Singer and Chernoff 1952 Singer and Fisher 1952 1953b) Itano (195) and Chernoff (1953) have demonstrated the presence of an alkali resistant (F or fœtal type) hæmoglobin in the erythrocytes of many patients with sickle cell anæmia This abnormal hæmoglobin is in all probability identical with normal fœtal (F) hæmoglobin (Itano 1953c Chernoff 1953) In carriers of the sickle cell trait F hæmoglobin can be detected in minute amounts (Chernoff 1953) or may be absent altogether (Beaven and White 1953)

Singer and Fisher (1952) reported the results of an analysis of 87 patients who gave unquestionable clinical and hæmatological evidence of sickle cell anæmia the erythrocytes of three patients contained no

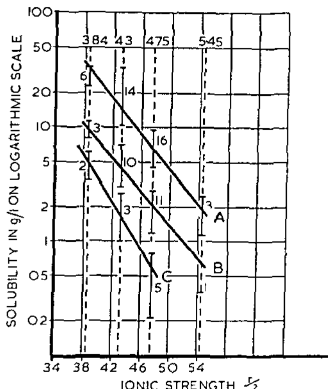


FIG 60 Solubility of reduced hæmoglobin in pH 6 7 phosphate buffer Regression lines fitted to experimental values for hæmoglobin derived from normal adult blood (A) from sickle cell trait blood (B) and sickle cell anæmia blood (C)
The range of values for each group at different ionic strengths is shown by the vertical bars The figures indicate the number of observations in each group (From White and Beaven 1954)

globin required for gelling and type C hæmoglobin reduced this still further Singer and Singer made the point that sickling is caused not only by the presence of S hæmoglobin but also by its interaction with other hæmoglobins

The Differentiation of Hæmoglobins S C and D The three types of abnormal hæmoglobin can be readily differentiated by physico chemical means (Itano 1953a) Hæmoglobin S differs from normal (A) hæmoglobin in electrophoretic behaviour and in its diminished solubility hæmoglobin C differs electrophoretically from both hæmoglobin A and hæmoglobin S but has a normal or above normal solubility *(Itano 1953b) hæmoglobin D although*

Robin King and Jefferson 1948 Callender Nickel Moore and Powell 1949 Singer and Fisher 1952) The published curves of erythrocyte elimination indicate marked differences in the survival of the transfused cells A proportion of the cells disappear rapidly from the circulation other cells are eliminated far more slowly As already referred to Singer and Fisher (1952) correlated this difference in survival time with the relative amounts of S and F hæmoglobins present and found that the cells containing the greatest amount of F hæmoglobin survived the longest

It remains to be considered how and why the life span of the erythrocytes in sickle cell anæmia is diminished *in vivo* It is possible that the oxygen tension in the blood becomes sufficiently low in areas where the circulation is slowed for massive sickling to take place and that this results in actual impaction of the cells with subsequent vascular occlusion and ultimate lysis of the sickled cells This no doubt takes place sometimes and may well be responsible for the occasional thrombotic incidents which patients may experience during the course of their disease and for the ultimate shrinkage and fibrosis of the spleen which so frequently occurs Singer (1951) in a review considered that this mechanism could not explain all the phenomena of the disease satisfactorily let alone the continuing hæmolysis which may persist for long periods without there being any clinical evidence of thromboses He also pointed out that in the experiments of Reinhard Moore Dubach and Wade (1943) in the course of which patients with sickle cell anæmia breathed oxygen at high concentrations for long periods sickling *in vivo* was probably reduced in extent without the rate of hæmolysis being obviously slowed

Shen Fleming and Castle (1949) showed that two types of sickled cell might develop *in vitro* one type which would revert to the normal shape in the presence of oxygen and another type which appeared to have become irreversibly sickled Presumably the sickled cells seen in smears of the peripheral blood of patients are of the second irreversibly sickled type Watson (1948) and Shen Fleming and Castle (1949) made the additional point that the cells which underwent irreversible sickling were nearly all adult erythrocytes not reticulocytes they concluded that irreversibility was a late stage in the development of the sickling phenomenon It is highly probable that erythrocytes when irreversibly sickled last only a short time in the circulation of the patient It is known for instance that sickled cells are unusually sensitive to the effects of mechanical trauma *in vitro* (Shen Castle

F hæmoglobin by the method used in the others the proportion ranged from 2 to 24% They concluded as the result of transfusion experiments that the erythrocyte population of patients with sickle cell anæmia was probably composed of three fractions (1) cells containing S (sickle cell) hæmoglobin but little or no F (fœtal) hæmoglobin (2) cells containing both S and F hæmoglobins and (3) cells containing F hæmoglobin with little or no S hæmoglobin The corpuscles containing S hæmoglobin had the shortest survival when transfused to normal recipients and the greatest sensitivity to mechanical trauma *in vitro* and the cells containing the most F hæmoglobin the longest survival *in vivo* and the greatest resistance to trauma *in vitro*

These studies are of great interest even if their meaning is not clear at the moment It is obvious that the demonstration of a proportion of an alkali resistant hæmoglobin in the erythrocytes of a sufferer from sickle cell anæmia does not mean that he necessarily carries the trait for Mediterranean anæmia that is to say he has microdrepanocytic disease (see p 143)

It has been claimed that small amounts of fœtal hæmoglobin may be detected in the blood of patients suffering from a wide variety of both congenital and acquired blood diseases and that minute amounts may be present in the blood of normal healthy adults (Singer Chernoff and Singer 1951 Chernoff 1953) The amounts present are however usually considerably less than in sickle cell anæmia and it should be added perhaps that Beaven and White (1953) using several methods were unable to detect F hæmoglobin in normal adult blood There is no doubt however of the presence of F hæmoglobin in sickle-cell anæmia and in thalassæmia but the cause or causes of its persistence in excessive amounts in adult erythrocytes are at the moment obscure

Attempts have been made to differentiate between sickle cell hæmoglobin and normal hæmoglobin by immunological means One of the first attempts was carried out by Cardozo (1937) rabbits were immunized with blood containing sickle cells and the rabbit sera subsequently absorbed with normal blood No specific agglutinins for sickle-cells however could be demonstrated

Vecchio and Barbagallo (1950) immunized rabbits with different types of hæmoglobin Using the precipitin reaction they failed to demonstrate any antigenic difference between normal and S hæmoglobin however they did find a difference between normal and F hæmoglobin as had been previously demonstrated by Darrow Nowakovsky and Austin (1940) Chernoff's (1953) results were essentially the same as those of Vecchio and Barbagallo Goodman and Campbell (1953) on the other hand using anti sera prepared in chickens have reported that clear differences in the specificity of normal and S hæmoglobin can be demonstrated if cross reaction tests are carried out quantitatively under optimum conditions They suggested that the two different hæmoglobins share many common antigenic determinants but that a small number are unique for each type

Pathogenesis of Sickle cell Anæmia

It has been clearly established that the erythrocytes of patients with sickle cell anæmia have a diminished life span *in vivo* (Singer

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anæmic patients a transfusion may be a life saving procedure However when the hæmoglobin is maintained at a level of 7 ₅ g per 100 ml or more it would seem unwise to undertake periodic transfusion unless there is some special additional indication such as pregnancy

Splenectomy has been undertaken sporadically but the results have generally been disappointing According to Margolies (19₅1) the operation was first carried out by Hahn and Gillespie (1927) and by Stewart (1927) Shotton Crockett and Leavell (19₅1) reviewed the results of splenectomy in 24 cases The symptoms of fifteen patients became less severe and there was some improvement in their erythrocyte counts of the others four patients improved slightly and four were not benefited The best results seem to have been obtained in patients who had the largest spleens i e when the operation was undertaken at a relatively early stage of the disease Dickerstein and Koop (quoted by Margolies 19₅1) for instance carried out splenectomy in 16 children ranging from 14 months to six years of age and followed their progress for one to four years after operation Two patients were not improved but the other fourteen did relatively well their hæmoglobins were maintained at slightly higher levels than before operation and none had had a major crisis since splenectomy

A C T H Sass (19₅2) reported dramatic symptomatic improvement when a patient suffering from sickle cell anæmia was given A C T H However no significant changes took place in the blood count and it is doubtful whether A C T H or cortisone has any real place in the palliative treatment of the disease

Prognosis The outlook for a patient suffering from sickle cell anæmia is grave and many sufferers die in the first decade Others reach adult life only to die of complications such as heart failure or of intercurrent infections Pregnancy is a severe hazard and the maternal and fœtal mortalities are relatively high (Beacham and Beacham 1950 Margolies 1951) As already referred to the outlook for patients suffering from variants of sickle cell disease such as sickle cell thalassæmia and sickle cell hæmoglobin—hæmoglobin C disease is more favourable than in true sickle cell anæmia

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and Fleming 1944) and it is likely that this is an important mechanism causing destruction *in vivo*

There is also evidence that chemical changes take place rapidly when corpuscles become sickled Tosteson Shea and Darling (1952) showed that in the de oxygenated state sickled cells quickly lost major amounts of potassium and gained substantial amounts of sodium Sickle trait cells on the other hand behaved almost normally These changes are probably due to the alteration in the physical state of the hæmoglobin and to the assumption of the sickled form It seems likely too that the marked cation changes are a reflection of actual damage to the cell membrane If this is so this work then provides evidence for an additional and perhaps all important mechanism of cell destruction

The crises of hæmolysis which occur from time to time and which are associated with abdominal pain and other symptoms remain unexplained as a rule there appears to be no concomitant depression of erythropoiesis In this respect the pathogenesis of these minor crises differs from the more serious aplastic crises which occasionally develop

There seems to be no evidence that auto antibody formation commonly plays a significant part in the causation of hæmolysis in sickle cell anæmia The work of Schneider and Levin (1950) who claimed to find abnormal agglutinins in 13 patients with sickle cell anæmia does not seem to have been confirmed

Treatment of Sickle cell Anæmia

Although nothing can be done to remedy the fundamental defect of hæmoglobin and erythrocyte formation in sickle cell anæmia palliative measures need some consideration A good account is given by Margolies (1951) Oxygen therapy the possible use of vasodilator drugs in the treatment of abdominal crises transfusion splenectomy and A C T H will be briefly mentioned

Oxygen therapy seems to be contraindicated Reinhard Moore Dubach and Wade (1944) concluded that prolonged administration *of oxygen did not inhibit hæmolysis and did not relieve pain* Moreover it inhibited compensatory erythropoiesis to some extent with the result that the patients became more anæmic

Vasodilator drugs such as Priscoline have been used in the treatment of abdominal pain on the hypothesis that the pain was due to vascular spasm Smith Rosenblatt and Bedo (1953) reported good results in seven children

Blood transfusions are of temporary value and in severely

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liver It was in France too that the first observations were made that suggested that hæmolytic anæmia might be caused by the development of auto antibodies From 1908 onwards Widal Abrami and Brulé (1908a and b 1909) in a series of papers gave the first accurate descriptions of l ictère hémolytique acquis Significantly they stressed that autohæmagglutination was characteristic of the cases they studied Other important observations were made in France at about the same time Chauffard and Troisier (1908) and Chauffard and Vincent (1909) described as suffering from ictère hémolysinique and hémoglobinurie hemolysinique patients in whom intense hæmolysis was taking place *in vivo* and whose sera appeared to contain abnormal hæmolysins

These pioneer studies were to some extent forgotten in the succeeding decades and it is only in comparatively recent years that their importance has been recognized and new advances made In 1938 Dameshek and Schwartz (1938a) again reported the presence of abnormal hæmolysins in patients suffering from acute (acquired) hæmolytic anæmia They also showed clearly both in man and in animals that spherocytosis and increased osmotic fragility might develop during the course of acquired hæmolytic anæmia (Dameshek and Schwartz 1938b) Later they published a comprehensive review in which was summarized almost all that was known about acquired hæmolytic anæmia up to that time (Dameshek and Schwartz 1940)

In 1945 there appeared a most important publication Coombs Mourant and Race showed that erythrocytes sensitized by incomplete forms of Rh iso antibodies were agglutinated by anti human globulin sera prepared by immunizing rabbits against human serum proteins This discovery provided a new tool in immunological research It was soon applied to the investigation of cases of hæmolytic anæmia In 1946 Boorman Dodd and Loutit and Loutit and Mollison reported that the direct anti globulin reaction (Coombs s test) was positive in a number of patients suffering from idiopathic acquired hæmolytic anæmia whilst the test was negative in patien s suffering from congenital and other types of hæmolytic anæmia These observations have since been confirmed in many parts of the world In 1947 Morton and Pickles reported that enzymes such as trypsin increased the susceptibility of human erythrocytes to certain types of antibodies The trypsinized cell technique has also proved to be extremely useful Both methods have helped enormously in the under standing of the pathogenesis of acquired hæmolytic anæmia by

CHAPTER 7

ACQUIRED HÆMOLYTIC ANÆMIA

I IDIOPATHIC AUTO ANTIBODY TYPE

THE term *acquired hæmolytic anæmia* is descriptive of a number of disorders of probably different ætiology and pathogenesis In many instances the excessive hæmolysis seems to be due at least in part to the action of antibodies directed against the patients own erythrocytes In other cases however the pathogenesis is less obvious and in some patients it is quite unknown In this chapter and in succeeding chapters the various types of acquired hæmolytic anæmia will be dealt with in succession

An account will first be given of those types in which there is definite evidence of the formation of auto antibodies—the auto immune type In the majority of cases the ætiology of the disorder is unknown The most frequent form of the disease is therefore provisionally designated idiopathic in other patients the hæmolytic process is the sequel to some recognized disease such as virus pneumonia or is associated with some additional pathological process such as chronic lymphatic leukæmia or disseminated lupus erythematosus These secondary or symptomatic cases are probably less rare than was at one time thought

The auto immune types of hæmolytic anæmia may be classified as follows —

(*a*) Idiopathic acquired hæmolytic anæmia
(*b*) Hæmolytic anæmia following virus pneumonia and certain other infections (see Chapter 8)
(*c*) Paroxysmal cold hæmoglobinuria (see Chapter 10)
(*d*) Hæmolytic anæmia associated with chronic lymphatic leukæmia reticulosarcoma or disseminated lupus erythematosus etc (see Chapter 13)

IDIOPATHIC ACQUIRED HÆMOLYTIC ANÆMIA

History *Hayem (1898) is generally credited with giving the* first recognizable description of acquired hæmolytic anæmia under the title Ictère infectieux chronique splénomegalique and of differentiating anæmia with jaundice from disease of the

Race and Inheritance As far as is known idiopathic acquired hæmolytic anæmia is not confined to any particular race or races However nearly all the published case reports deal with patients of European origin It has generally been thought that there is no evidence for a genetic basis for the disease Kissmeyer Nielson Bent Hansen and Kieler (1952) however have published an account of a family in which both a mother and her daughter developed a hæmolytic anæmia of an auto immune type This observation is clearly exceptional but it is difficult to dismiss it as mere coincidence On the other hand one of the author s patients had an unaffected sister who was probably an identical twin (see p 197)

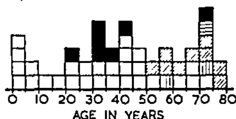


FIG 62 Age distribution of 47 patients suffering from acquired hæmolytic anæmia of the auto immune type

□ = warm antibody type
|||| = secondary warm antibody type
//// = cold antibody type
≡ = secondary cold antibody type
■ = cold antibody type after virus pneumonia

Age and Sex. Persons of all ages are affected as well as both sexes Sacks Workman and Jahn (1952) reviewing 147 cases of idiopathic and secondary acquired hæmolytic anæmia from the literature as well as 19 cases of their own found that two thirds of the patients were females In the author s series too more females than males have been affected 25 out of 37 patients suffering from the idiopathic type of the disease being females a proportion of females which differs significantly from one half There is no obvious association with pregnancy or parity

The disease is not congenital although it may occur in quite young infants The youngest patient the author has personally investigated was aged 5 months the oldest was aged 78 at the time of onset of the disease The distribution in five year periods of the age of onset of the patients studied by the author is illustrated in Fig 62 One variant of the disease (see p 175) in which

demonstrating the presence of incomplete antibodies in cases where other techniques had failed to do so

Synonyms L ictère hémolytique acquis (Widal Abrami and Brulé 1909) acquired hæmolytic splenomegalic icterus of the Hayem Widal type (Micheli 1911) acquired acholuric jaundice (Eason 1918) acute hemolytic anæmia (Lederer 1925 Dameshek and Schwartz 1940) immunohemolytic anæmia (Evans *et al* 1951) autoimmune hemolytic disease (Young Miller and Christian 1951)

Some continental workers (e g Marcolongo 1953) have referred to different forms of idiopathic acquired hæmolytic anæmia by the eponyms Hayem Widal ' Dyke Young Loutit and Lederer Brill whilst the term Lederer s Anæmia has been widely used in British and American literature While not disputing that wide differences exist among patients in respect of their clinical histories and in the results of laboratory tests the present author feels that it is unwise to attempt to separate a disease of such varying expression as acquired hæmolytic anæmia into many subgroups unless the subdivisions can be made on the basis of real differences in pathogenesis Some differentiation on these lines is possible and the clinical syndrome of acquired hæmolytic anæmia due to warm auto antibodies may for instance be differentiated in most cases from that due to " cold auto antibodies Even so the clinical pictures are less distinct than are the serological findings

One syndrome deserves separate consideration this is the acute hæmolytic anæmia of unknown origin to which Lederer s name has been attached Probably the eponym should be dropped altogether However the clinical syndrome of acute hæmolytic anæmia of short duration described by Lederer is to some extent distinctive Even so it is probably brought about by more than one mechanism and merges imperceptibly both clinically and pathologically into idiopathic acquired hæmolytic anæmia of a less dramatic type Nevertheless because of its rather distinctive clinical course and historical associations Lederer s anæmia will receive separate consideration

General Features of Idiopathic Acquired Hæmolytic Anæmia

Recent reviews include those of Dameshek and Schwartz (1940) Dreyfus Dausset and Vidal (1951) Young Miller and Christian (1951) Baumgartner (1952) Marcolongo (1953) Heilmeyer (1953) and Young and Miller (1953a)

antecedent illness Exceptionally signs and symptoms of thrombocytopenic purpura may have preceded or be associated with those of hæmolytic anæmia (see p 178)

Young and Miller (1953b) emphasized how in some patients repeated attacks of hæmolysis might be followed by spontaneous remissions One patient for instance suffered from six episodes of acute hæmolytic anæmia within four years of the initial attack for which splenectomy had been performed

Physical Signs *Anæmia* The degree of anæmia varies from mild to extremely severe On the whole patients with idiopathic acquired hæmolytic anæmia tend to be more anæmic and are generally more seriously ill than are patients suffering from hereditary spherocytosis The minimum hæmoglobin concentrations and erythrocyte counts and other hæmatological data in a small series of patients intensively studied by the author are recorded in Table 6

Jaundice Usually the patient is visibly jaundiced to a moderate degree The hyperbilirubinæmia is due as a rule solely to an excess of pigment which gives a positive indirect van den Bergh reaction the jaundice is thus typically acholuric in nature In seriously ill patients however the direct reaction may be positive and bile pigment may also appear in the urine This is probably due to actual liver damage focal areas of necrosis being not uncommonly found in fatal cases (see p 180)

Splenomegaly The spleen is probably always considerably enlarged varying according to Dameshek and Schwartz (1940) from one and a half to five times its normal size Usually it is readily palpable however it may not be felt at the onset of an acute attack It is unusual for an enlarged spleen to reach the umbilicus Sometimes the spleen is tender on palpation especially is this true in acute hæmolytic episodes

Other Physical Signs The *liver* is often slightly enlarged particularly in the most anæmic patients The other organs of the body appear to be essentially normal on physical examination except for the effects that anæmia may have on them Enlargement of lymph nodes does not usually occur nor is purpura commonly found

Urine A moderate excess of urobilinogen is generally found and occasionally bile pigments also In some cases actual hæmoglobinuria may occur In seriously anæmic patients there may be slight albuminuria and a few casts may be found in the urinary deposit Hæmosiderin is also frequently found in small amounts (Crosby and Dameshek 1951)

cold antibodies are present in very high concentrations seems to be most commonly found in elderly subjects (Ferriman Dacie Keele and Fullerton 1951)

Incidence Acquired hæmolytic anæmia of the auto antibody type is an uncommon but not a rare disease Sacks Workman and Jahn (1952) collected 147 cases published in the literature between 1940 and 1951 and added 19 patients of their own in 85 patients the anæmia was secondary to some underlying disease The author has investigated 49 patients with acquired hæmolytic anæmia in a seven year period However only fourteen of these patients were in patients in Hammersmith Hospital a general hospital of about 600 beds Some of the remaining patients studied by the author have been attending other London hospitals other patients have been in hospitals in various parts of the country and serological studies were carried out on samples of their blood sent by post Ten of the patients have suffered from secondary acquired hæmolytic anæmia in seven instances this followed virus pneumonia Thus the disease was apparently idiopathic in 39 patients in 30 cases the auto antibodies were of the warm type or were predominantly of the warm type in nine patients the antibodies were of the cold type

Clinical and Hæmatological Features

1 Warm Antibody Type

Symptoms of the Disease Idiopathic acquired hæmolytic anæmia is a most variable disorder and almost every grade of severity may be met with In some patients the illness may be a chronic one extending over years and the only symptoms complained of may be those common to chronic mild anæmia of any cause e g undue tiredness and mild dyspnœa on exertion In more severely affected patients the severity of the anæmia often leads to serious dyspnœa and incapacity Sometimes chronic jaundice may be the patient s chief complaint but this is a very variable symptom In the most severely affected patients the onset may be very sudden instead of being insidious the chief features of the disease being rapidly increasing anæmia and increasing jaundice often accompanied by pyrexia and a shock like prostration In these cases too hæmoglobinuria may be noticed and the title acute hæmolytic anæmia is more than justified Occasionally the onset of the anæmia may seem to have followed an infection of some kind but in most cases it appears spontaneously without apparent cause or recognizable

average count was 20% (13 patients) the range being 1% to 67% (Table 2 p 21)

Sometimes auto agglutination may be obvious even in well made films prepared from freshly drawn blood When present this phenomenon is suggestive of the presence of auto antibodies It is most commonly seen when cold antibodies of high thermal amplitude are present (see Fig 66) it may however be observed occasionally with patients whose blood contains warm auto agglutinins Auto agglutination has to be distinguished from rouleaux formation This as a rule presents no difficulty as the distribution and shape of the agglutinated masses of cells in true agglutination is quite distinct from that in rouleaux formation

Erythrophagocytosis usually by monocytes may occasionally be seen in fresh preparations of peripheral blood (Fig 64) Recent reports describing this phenomenon include those of Hargraves Herrell and Pearman (1941) Landolt (1946) and Gasser and Hollander (1951) A good account of the incidence of erythro phagocytosis in hæmolytic disorders generally is given by Baumgartner (1948) All the patients whose blood pictures were described by the authors mentioned above were acutely ill in intense hæmolytic episodes More recently Zinkham and Diamond (1952) described erythrophagocytosis in the blood of two infants one suffering from an acute and the other a chronic idiopathic acquired hæmolytic anæmia Erythrophages were found in small numbers in fresh smears of peripheral blood they were present however in much larger numbers in smears made of the buffy coat of blood centrifuged after incubation for one half to two hours at 37 C (see also p 14)

Reticulocytes As in other types of chronic hæmolytic anæmia a persistently raised reticulocyte count is a characteristic finding sometimes the count may exceed 50% As mentioned above the reticulated cells are generally conspicuously macrocytic as compared with fully ripened cells Naturally at the onset of a hæmolytic episode in a previously healthy subject the reticulocyte count may be within the normal range Aplastic crises during the course of an idiopathic acquired hæmolytic anæmia are rare but have been described (Davis Kennedy Baikie and Brown 1952)

Leucocytes The total leucocyte count varies within wide limits in idiopathic acquired hæmolytic anæmia Often in chronic cases the count (particularly the neutrophil count) is low (Table 6) the cause of this is not known with certainty In acute hæmolytic episodes however it is quite common for the leucocyte count to be raised to 30 000 cells per c mm or even higher chiefly due to

Fæces An increased fæcal excretion of urobilinogen is the rule and as in congenital hæmolytic anæmia the daily total pigment excretion may exceed 1 000 mg

The Blood Picture

Erythrocytes The anæmia is more often than not macrocytic rather than normocytic as judged by mean corpuscular volume measurements (Table 6) The *macrocytosis is regenerative in* nature the macrocytes being derived from normoblasts (macro normoblasts) rather than from megaloblasts (see Dacie and White 1949) Cell diameter measurements nevertheless may also reveal microcytosis this is due to the presence of microsphero cytes These acquired spherocytes are more or less con spicuous in most cases when there is active hæmolysis In hyperacute cases spherocytosis may be extremely marked (Fig 14) Occasionally no spherocytes can be found particularly is this so in patients in remission (see Young and Miller 1953a) The mean corpuscular hæmoglobin concentration is normal or may be raised in patients in whom there is a marked degree of spherocytosis (cf hereditary spherocytosis Table 1 p 17)

There is usually a considerable degree of anisocytosis If there is marked spherocytosis the differences in cell diameters of the cell population are often striking the microspherocytes being as small as 5μ and the flattened macrocytes as large as 10μ The latter often stain diffusely basophilic for the largest cells are generally reticulocytes The contrast in cell diameters and staining *is illustrated in Figs 1 and 13 (pp 12 and 18)* Punctate basophilia may be present but is not usually a marked feature Poikilo cytosis is not as a rule conspicuous the spherocytes in particular having a notably rounded contour

Normoblasts are often present in peripheral blood smears but they are only present in large numbers in cases where hæmolysis is extremely rapid Probably they always increase in number after splenectomy if the operation does not reduce the rate of hæmolysis (Fig 14 p 18) The normoblasts are usually poly chromatic forms

Siderocytes are present in small numbers in the peripheral blood of some cases of acquired hæmolytic anæmia (before splenectomy) Douglas and Dacie (1953) studied 19 patients the largest numbers of siderocytes were found in patients in whom hæmolysis was most intense the average count was 2 3% with a range from 0 to 21% After splenectomy the

an increase in neutrophils and for metamyelocytes and myelocytes to be present in the circulation

Platelets The platelet count is normal or low in idiopathic acquired hæmolytic anæmia in some cases thrombocytopenia may be marked and be accompanied by clinical purpura (see p 178)

Osmotic Fragility

There is usually a moderate increase in osmotic fragility corresponding with the degree of spherocytosis seen in peripheral blood films It seems probable that osmotic fragility is nearly always but not quite invariably increased in patients in whom hæmolysis is active In patients in complete remission on the other hand the fragility is likely to be normal or at the most only very slightly increased A greatly increased osmotic fragility is always associated with a serious degree of hæmolysis (e g cases 11 and 12)

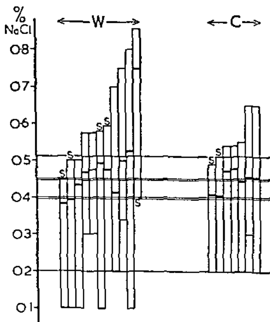


Fig 63 Results of osmotic fragility tests in eleven patients with acquired hæmolytic anæmia of the warm antibody type (W) and seven patients with acquired hæmolytic anæmia of the cold antibody type (C) The horizontal lines indicate the normal range and the double lines the normal range of the M C F S denotes after splenectomy (cf Fig 31)

TABLE 6 *Hæmatological Data in Seven Patients suffering from Idiopathic Acquired Hæmolytic Anæmia*

Case No. and Type of Disease	Erythrocytes (minimum) milli per c mm	Hæmoglobin (minimum) g per 100 ml	M C V (average) c μ	M C H C (average)	Reticulocytes (maximum)	Leucocytes per c mm	Platelets per c mm	Serum bilirubin (maximum) mg per 100 ml
8–12 Idiopathic warm antibody type	1 3–2 4	5 3–8 7	103–124	33–42	15–36	3 000– 3 000	170 000– 270 000	2 0–5 0
13 Idiopathic cold antibody type (without Raynaud's phenomena)	2 1	8 7	113	34	8 3	1 800– 5 000	21 000– 67 000	1 4
14 Idiopathic cold antibody type (with Raynaud's phenomena)	1 8	6 2	109	34	16	1 900– 8 000	50 000– 150 000	2 5

by the normal amount by glucose (Selwyn and Dacie 1954) and acquired spherocytes

Clinical and Hæmatological Features
2 Cold Antibody Type

As already mentioned in a minority of patients suffering from idiopathic acquired hæmolytic anæmia the auto antibodies are of the cold type In some instances the presence of the cold anti bodies in high concentrations produces a rather characteristic clinical syndrome characterized by Raynaud s phenomena a chronic often relatively mild hæmolytic anæmia and episodes of hæmoglobinuria However not all patients with hæmolytic anæmia of the cold antibody type present these distinctive clinical features In two patients investigated by the author the clinical course was indistinguishable from chronic acquired hæmolytic anæmia of the warm antibody type in a third the disorder presented itself as an acute hæmolytic episode with hæmoglobinuria which responded dramatically to splenectomy The clinical history and pathological findings of one of these patients are described on p 205 (Case 13) In none of these patients were the cold antibodies present in such high concentrations as in the patients exhibiting Raynaud s phenomena (Table 8 p 203)

The chief clinical and hæmatological features of the type with Raynaud s phenomena were reviewed by Ferriman and co workers (1951) who also described three personally studied cases Other reports in the literature include those of Roth (1935) Salén (1935) Ernstene and Gardner (1935) McCoombs and McElroy (1937) Benians and Feasley (1941) Stats and Bullowa (1943) Whittle Lyell and Gatman (1947) Heilmeyer Hahn and Schubothe (1947) Malley and Hickey (1949) van Loghem Mendes de Leon Frenkel Tietz and van der Hart (1953) Nelson and Marshall (1953) and Heilmeyer (1953)

Age and Sex The disease seems particularly to affect rela tively elderly subjects the ages of the patients so far described having ranged from 40 to 78 years (see Fig 62) Both sexes have been affected

Symptoms of the Disease Cyanosis and Raynaud s pheno mena are characteristically produced by exposure to cold The patients fingers toes hands feet and sometimes nose and ears become at first white and then purplish blue in colour Occa sionally too actual gangrene of a digit has been observed (Ferri man *et al* 1951) These Raynaud s phenomena are brought about

Observations on a series of patients recently investigated by the author are summarized in Fig 63 The complex relationship between antibody action and increase in osmotic fragility is considered in a later section (p 302)

Osmotic Fragility after 24 Hours' Incubation at 37 C

Increases in osmotic fragility often but not invariably greater than normal are produced by incubating the blood of patients with acquired hæmolytic anæmia for 24 hours at 37 C Selwyn (1953) studied 5 cases in three of them the increase in osmotic fragility exceeded that of incubated normal blood It seems unlikely however that study of the changes in fragility produced by incubating blood from patients with acquired hæmolytic anæmia will have the practical application in diagnosis that it has in hereditary spherocytosis (see p 63) Young and Miller (1953a) also found that the increases in osmotic fragility were less regular than in hereditary spherocytosis

Autohæmolysis (Incubation at 37 C for 24 to 48 Hours)

The rate of autohæmolysis of blood from patients suffering from acquired hæmolytic anæmia is usually significantly increased (Dacie 1950a) Occasionally lysis occurs extremely rapidly so much so that in two of the author s cases (Cases 11 and 12) visibly increasing lysis was obvious within an hour or so of collection and in one patient it proved impossible to obtain unhæmolysed serum or plasma It is undoubtedly significant that in both these patients there was an extreme degree of spherocytosis It is in fact probable that the lysis was due not so much to an immune body reaction involving complement as to the disintegration of markedly spherocytic cells (see p 303) Young Izzo and Platzer (1951) also referred to the very rapid lysis of the erythrocytes of two patients in hæmolytic crises they contrasted this with the rates of lysis only just above the normal observed in the same patients during quiescent phases of their disease

Selwyn (1953) studied the effect on hæmolysis of maintaining a high concentration of glucose throughout the incubation period In four out of five patients glucose had less than its normal effect in diminishing hæmolysis and in one severely ill patient with marked spherocytosis and a rapid rate of autohæmolysis the presence of glucose had absolutely no effect in diminishing hæmolysis In this respect therefore there are differences between congenital spherocytes the autohæmolysis of which is diminished



Fig 64 Photom crograph of a l lood film of a p atient suffering from hyperacute idiopathic ac quired hæmolytic an mia (Case 12) There is intense sp herocyto is auto agglutination an l erythro phagocyto i × 700

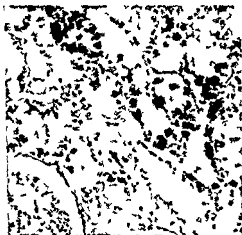


Fig 6 S dero is of th e kidney of a patient suffering from acquired hæmolyt c anæm a of th e cold antibody type (Ca e 9 of Ferriman et al 19 1) Perl s react on × 175

by auto agglutination of the patients erythrocytes taking place in peripheral blood vessels as the result of chilling (Marshall Shepherd and Thompson 1953)

Most patients experience hæmoglobinuria in particularly cold weather The frequency of hæmoglobinuria however varies from patient to patient In some such as the patient described as Case 2 by Ferriman and co workers (1951) no hæmoglobinuria was ever observed despite the fact that Raynaud s phenomena were intense in cold weather in others such as the patient described by Bonnin (1954) hæmoglobinuria was a very striking symptom It is possible that the differences in the incidence of hæmoglobinuria can be correlated with differences in the hæmolytic potency of the antibodies (see p 309) It is interesting to note that in patients not developing clinical hæmoglobinuria intravascular hæmolysis nevertheless probably takes place to some extent as shown by the intense renal siderosis which may be found (Fig 6a)

The patients are usually visibly jaundiced but only in a minority has the spleen been palpable

The disease is usually a very chronic one and is relatively benign One of the patients described by Ferriman and co workers (1951) was known to have been affected since 1938 The anæmia is as a rule not very severe In only two out of the twelve patients mentioned by Ferriman and co workers (1951) was the hæmoglobin concentration reported as falling below 7 4 g per 100 ml Aside from the Raynaud s phenomena it appears likely that the intensity of hæmolysis and the degree of anæmia are directly related to temperature most patients being more anæmic in winter time The clinical history of a hitherto unreported example of this type of hæmolytic anæmia is given on p 208 (Case 14)

Blood Examination Intense auto hæmagglutination *in vitro* is a characteristic feature of the disease Usually the blood of the patient undergoes gross clumping immediately after withdrawal unless its temperature is maintained strictly at 37° C It is this feature which has often called attention to the disease in the first place Associated with the clumping there is a tendency for concentrated cell serum suspensions to undergo hæmolysis as stressed by Stats and Wasserman (1943) and unless care is taken it is difficult to obtain unhæmolysed plasma or serum If however the blood is delivered by means of a needle and a short piece of rubber tubing into a container previously warmed to 37° C unhæmolysed plasma or serum may be regularly obtained Similarly good blood films may be made if slides previously warmed at 37 C are used Films made on slides not warmed

above room temperature usually present the characteristic appearance shown in Fig 66

Except for the tendency to autohæmagglutination blood films show as a rule no very striking features though there may be slight macrocytosis Polychromasia will be present in accordance with the reticulocyte count Spherocytosis is usually not conspicuous and in most of the reported cases the osmotic fragility has been reported as normal that of Case 14 was however definitely although slightly increased (see also Fig 63) The hæmatological data on Case 14 are summarized in Table 6 (p 172)

Other Findings *Urine* Hæmoglobinuria developing occasionally as the result of exposure to cold has already been referred to Hæmosiderin if looked for would probably be more constantly observed judged by the degree of siderosis of the kidneys found at postmortem (Fig 65)

Wassermann and Kahn Reactions These have been uniformly reported as negative

The *serological findings* in the cold antibody type of idiopathic acquired hæmolytic anæmia are described on p 194

Pathology is considered on p 179 and *Treatment* on p 314

Clinical and Hæmatological Features
3 Lederer's Anæmia

In 1925 Lederer described three patients who had suffered from acute hæmolytic episodes of sudden onset and of short duration In each case recovery seemed to take place following a blood transfusion Brill (1926) reported what might well have been a similar type of case Subsequently Lederer (1930) described three additional patients Later the use of the term Lederer's anæmia became widespread in medical literature

Lederer's cases comprised three adults and three children in each patient the onset of the disease was sudden and within three to six days they became jaundiced and profoundly anæmic The sudden hæmolytic crises were associated with fever headache vomiting and prostration Two of the patients had marked hæmoglobinuria and one became semi comatose All of Lederer's patients had high leucocytoses of between 33 000 to 81 000 cells per c mm Myelocytes were present in the peripheral blood and also many normoblasts Osmotic fragility (when estimated) was found to be normal or almost normal

Lederer's patients were transfused at the height of their anæmia and all recovered and became quite normal subsequently Lederer considered that recovery was initiated by transfusion and that this was a life saving procedure but admitted (Lederer 1930) that less severe cases might recover spontaneously

Since Lederer's papers many other cases have been described in

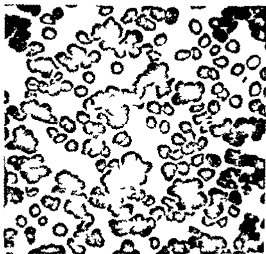


Fig. 66. Photomicrograph of a blood film of a patient suffering from *idiopathic acquired hæmolytic anæmia of the cold antibody type* (Case 3 of Ferriman *et al.*, 1951). × 400

The thesis that the anæmia thrombocytopenia and leucopenia might have a pathogenesis in common was further developed by Evans and co workers (1951) Out of 18 patients with acquired hæmolytic anæmia ten had normal platelet (and leucocyte) counts four patients had thrombocytopenia but no purpura and four had thrombocytopenia and the clinical signs of purpura (one patient had tuberculosis of the spleen) In addition it was reported that in six out of eleven patients with thrombocytopenia but without hæmolytic anæmia the direct antiglobulin test was never theless positive Two of the patients of Evans and co workers suffering from hæmolytic anæmia and purpura were women both of whom were pregnant in these patients and in a male patient the hæmolytic anæmia and purpura seem to have developed simul taneously Another (fatal) instance of simultaneous hæmolytic anæmia and thrombocytopenic purpura was described by Gasser and Hollander (1951) in an infant

Acquired hæmolytic anæmia has also supervened in patients who had previously been known to suffer from or had even undergone splenectomy for thrombocytopenic purpura Waugh (1932) described an example of this sort in a woman aged 39 who died of a fulminating hæmolytic anæmia Four years previously she had undergone splenectomy for chronic purpura The patient described by the present author as Case 12 (p 202) and Case 10 of Dacie and de Gruchy (1951) are further examples of acquired hæmolytic anæmia developing in patients previously splenecto mized for thrombocytopenic purpura At least two additional patients (Cases 13 and 14) are known to have had low platelet and neutrophil counts neither however developed spontaneous purpura Other patients known to have leucopenia and thrombo cytopenia were described by Young and Miller (1953b)

Pathology of Idiopathic Acquired Hæmolytic Anæmia

Bone marrow As in other chronic hæmolytic anæmias hyper trophy of the bone marrow takes place to a varying extent This is roughly proportional to the intensity of the hæmolytic process Thus red marrow spreads into the shafts of the long bones where in adults little hæmopoiesis normally takes place The fat spaces normally present may almost if not entirely disappear This hypertrophy is primarily due to hyperplasia of erythropoietic cells with the result that the erythroid myeloid ratio may even exceed unity Bone marrow biopsy shows that erythropoiesis is normoblastic or macronormoblastic in type

which an acute hæmolytic process ran a self limited course and in some blood transfusion has seemed to initiate recovery Most of the patients have been children (Patterson and Stewart Smith 1936 Giordano and Blum 1937 Greenwald 1938 Baxter and Everhart 1938 Betke Richarz Schubothe and Vivell 1953 etc) However it is now clear that although recovery is usually rapid this may take place more slowly and that lack of a dramatic response to transfusion does not necessarily mean that complete recovery will not take place (Fisher 1947)

It is difficult to make any absolute distinction between Lederer s hæmolytic anæmia and acute subacute and milder cases of idiopathic acquired hæmolytic anæmia Possibly if the term Lederer s anæmia is to be retained at all it should be only used to describe cases of acute onset and short duration going on to complete recovery Dameshek and Schwartz (1940) in their review included Lederer s anæmia as a form of acute hæmolytic anæmia They pointed out that cases probably identical with those of Lederer had been described previously and gave as early examples those of Chauffard and Vincent (1909) and Nobel and Steinebach (1914)

Recent work on the serology of Lederer s anæmia (see p 190) has demonstrated in some patients at least an auto immune mechanism for the anæmia identical with that usually found in the more chronic cases of idiopathic acquired hæmolytic anæmia In some patients however no abnormal antibodies can be demon strated by the techniques now available This type of case is referred to again in Chapter 14 p 357 The *ætiology* of acute hæmolytic anæmia is discussed in Chapter 12

Relationship between Acquired Hæmolytic Anæmia Thrombocytopenia and Thrombocytopenic Purpura

Particularly since the publications of Evans and Duane (1949) and Evans Takahashi Duane Payne and Liu (1951) especial interest has been taken in the relationship between thrombo cytopenia and acquired hæmolytic anæmia of the auto antibody type Evans and Duane (1949) reported that five out of eleven patients suffering from acquired hæmolytic anæmia had per sistently low platelet counts and that one of the patients actually suffered from clinical manifestations of purpura In two of the patients there was a symptomless leucopenia It was suggested that the thrombocytopenia and leucopenia might depend upon the formation of auto antibodies capable of destroying platelets and leucocytes in addition to those acting upon the patients erythrocytes

necrosis is not fully understood One possible factor is autohæmagglutination leading to circulatory stasis and consequent anoxia

Kidneys In fatal cases a variable degree of tubular damage may be seen Usually there is a moderate amount of siderosis this may be a very striking feature in patients in whom the plasma hæmoglobin concentration is constantly raised with or without hæmoglobinuria (Fig 65) Most of the hæmosiderin is in the loops of Henle and the second convoluted and collecting tubules In patients who have died with hæmoglobinuria pigment containing casts may be conspicuous in the collecting tubules

Lymph Nodes These are not usually significantly enlarged and their basic histological structure is normal However an unusual intensity of erythrophagocytosis by free phagocytic cells in the lymph sinuses may be observed

Other Organs No specific changes are encountered The usual effects of anæmia will be present in addition to a variable degree of siderosis the latter depending to a great extent on the history of the patient in respect of blood transfusion

BLOOD TRANSFUSION STUDIES IN ACQUIRED HÆMOLYTIC ANÆMIA

The first accurate studies in erythrocyte survival after transfusion of normal blood to cases of hæmolytic anæmia seem to have been carried out by Dacie and Mollison (1943) using the Ashby method In this paper the point was made that the unimpaired survival of normal erythrocytes in patients suffering from hereditary spherocytosis was in strong contrast to that observed (by Mollison) in a series of patients with acquired hæmolytic anæmia of varied type in which the destruction of normal corpuscles was rapid and usually complete within 20 days of the transfusion In this latter series were five patients suffering from acquired hæmolytic anæmia of the idiopathic type Brown Hayward Powell and Witts (1944) studied two patients with acquired hæmolytic anæmia The average life span of the normal erythrocytes after transfusion to these patients was calculated to be 7 8 and 13 1 days respectively It was found that if the number of surviving normal corpuscles was plotted against time the course of elimination formed a curve being at first rapid and then progressively less rapid in contrast to the almost straight line type of elimination found in normal subjects (see Fig 22 p 35) It was suggested that the curved form of the graph of elimination indicated that an exponential hæmolytic mechanism was at work which resulted in the destruction of the erythrocytes indiscriminately irrespective of their age

In some cases evidence of erythrophagocytosis by fixed phagocytic cells may be seen in sections of bone marrow. Erythrophagocytosis is not, however, commonly seen in films of material aspirated by marrow puncture; this is probably because the fixed phagocytic cells, if aspirated at all, remain embedded in fragments of marrow tissue. The amount of iron detectable by Perls's reaction is as a rule small, no doubt as a consequence of the rapid re-utilization of iron in the synthesis of fresh hæmoglobin.

Spleen. Early reports of the histology of the spleen in acute hæmolytic anæmia are summarized by Dameshek and Schwartz (1940). The organ has usually been reported to be between twice and five times enlarged. The histological picture is not as uniform as it is in patients with hereditary spherocytosis. However, there is usually considerable congestion with blood and sometimes this approaches in degree that seen in hereditary spherocytosis. Dameshek and Schwartz (1940) mentioned the presence in one of their cases of numerous thromboses of veins and capillaries which resulted in multiple infarctions. Sometimes macroscopic infarcts occur.

Irrespective of the degree of congestion, there is hyperplasia of the reticulum cells of the spleen pulp; in most instances, too, erythrophagocytosis is easily seen. Some of the erythrophages are distended with up to six or even more ingested erythrocytes; in other cells abundant brownish iron-containing pigment (hæmosiderin) is evidence of past phagocytic activity. In these respects —reticulum-cell hyperplasia and erythrophagocytosis—the histological appearances of the spleens differ from those of typical hereditary spherocytosis in which neither reticulum-cell hyperplasia nor evidence of phagocytosis is well marked. Another commonly observed feature is the presence of small islands of myeloid (mostly erythroid) metaplasia. Again this change is not commonly seen in hereditary spherocytosis.

Liver. In fatal cases the liver has usually been described as enlarged. The enlargement is mostly due to congestion with blood. There may in addition be areas of focal necrosis as well as hyperplasia of Kupffer cells. Sometimes small islands of erythropoiesis can be detected. Siderosis is often a striking feature, the iron-containing granules being present both in Kupffer cells and in liver parenchyma cells. The intensity of siderosis depends to a great extent on the number of times the patient has been transfused during life.

Occasionally an acute hæmolytic process is accompanied by signs of serious liver damage (Farrar, Burnett and Steigman, 1940); the patient may then become quite deeply jaundiced and have bile in the urine. In these patients a serious degree of liver-cell necrosis probably occurs. It should be added that the sequence of events leading to

from the patient's cells *in vivo* and transference to the recipient's erythrocytes as well as to the fact that the patient's corpuscles are no longer exposed to further sensitization when circulating in a normal environment

Owren (1949) also studied the survival of the sensitized corpuscles of a patient suffering from idiopathic acquired hæmolytic anæmia when transfused to a normal recipient as well as that of normal erythrocytes transfused to the patient The normal erythrocytes were quickly destroyed half being eliminated in six to seven days As in Selwyn and Hackett's experiments the elimination of the patient's corpuscles took place in two phases first a rapid fall within three days to about 35% of the immediate post transfusion count and then a much slower elimination which was still incomplete 80 days after the transfusion Owren found that the transfused (patient's) corpuscles gave positive antiglobulin tests during the first three days after transfusion at the time when the corpuscles were being rapidly eliminated

The studies referred to above clearly demonstrate that normal erythrocytes are destroyed unusually rapidly after transfusion to patients suffering from acquired hæmolytic anæmia they give also an approximate idea of the rapidity with which the patient's own corpuscles are probably being destroyed Survival curves of normal corpuscles transfused to one of the author's patients (Case 13) are illustrated in Fig 22 (p 85) In this patient clinical cure followed splenectomy and there was a corresponding improvement in the survival of normal corpuscles after transfusion

✓SEROLOGY OF IDIOPATHIC ACQUIRED HÆMOLYTIC ANÆMIA

In this section will be reviewed some of the more important general aspects of the auto antibodies of idiopathic acquired hæmolytic anæmia A more detailed consideration of the nature of the antibodies their properties and their behaviour *in vitro* is given in Chapter 9

The most important single fact about the abnormal antibodies developed by patients with acquired hæmolytic anæmia is that they are auto antibodies i e they are capable of being adsorbed by and of causing damage to the patient's own erythrocytes In addition the antibodies in most if not in all instances act on normal corpuscles also i e they act as iso antibodies as well as auto antibodies Until recently it was generally held that the antibodies were always non specific and acted on erythrocytes quite independently of their blood group or type Now it is known that in some cases the auto antibodies have a definite

Mollison s early observations mentioned by Dacie and Mollison (1943) were later reported in full by Loutit and Mollison (1946) and Mollison (1947) In Loutit and Mollison s paper eight examples of idiopathic acquired hæmolytic anæmia were referred to in seven of them the elimination of transfused corpuscles was very rapid it was relatively slow but still abnormal in one patient In some of the patients the graph of elimination was curved and roughly exponential in form In one patient the rate of elimination was much slower after splenectomy than before splenectomy Blood was withdrawn from four patients whose corpuscles gave positive direct antiglobulin (Coombs) tests and transfused to normal recipients Three of the patients who acted as donors were in clinical remission (two after splenectomy) the fourth patient had made a spontaneous and apparently complete recovery at the time the blood was withdrawn The survival of the erythrocytes of two of the patients was less than expected one week after transfusion (84% and 79% respectively) the subsequent rate of elimination however seemed to be strictly normal The erythrocytes of the other two patients were eliminated at the normal rate throughout

Mollison (1947) described observations made on five cases of idiopathic acquired hæmolytic anæmia Once again normal erythrocytes were shown to be rapidly destroyed after transfusion to the patients In each case elimination of half the transfused cells took place in six days or less in one patient 45% of the transfused blood was eliminated within the first nine hours of transfusion Other similar observations are given by Mollison (1951) In a patient recently studied who died of a hyperacute hæmolytic anæmia (Case 12 p 205) Dr Mollison found that normal corpuscles were destroyed almost as fast as they were transfused to the patient

Selwyn and Hackett (1949) carried out some interesting experiments They found that not only was there a markedly increased rate of elimination of normal corpuscles transfused to three patients suffering from idiopathic acquired hæmolytic anæmia but the normal corpuscles also became sensitized (as shown by their reaction with antiglobulin serum) in the recipients circulation before they were eliminated They also carried out the reverse procedure of transfusing the blood of patients into normal recipients In two experiments they found that the patients blood was eliminated at an increased rate for the first 10 to 15 days after transfusion thereafter elimination took place at the normal rate The transfused cells remained sensitized until their elimination It appeared however that antibody was also transferred from the patients to the recipients erythrocytes as the majority of the recipients corpuscles became sensitized after the transfusion as judged by their agglutination by antiglobulin serum

It seems likely that the relatively good survival of a patient s corpuscles in a normal recipient is due to elution of the antibody

anæmia is not the cause but may be the result of the anæmia However they added that the presence of the hæmagglutinin and hæmolysis *in vivo* might be associated pathogenetically in the acute hæmolytic anæmia following virus pneumonia which was just becoming recognized at about the time their review was published The majority of the reports concerned with auto hæmagglutination undoubtedly refer to cold agglutinins—some of these have already been referred to (p 175) Like Stats and Wasserman (1943) many of the authors were in doubt as to the significance of their observations Some of their observations were however noteworthy

Salen (1935) carried out extensive studies on the reactions *in vitro* of a very high titre cold antibody and Rosenthal and Corten (1937) and Reisner and Kallstein (1942) stressed the high thermal activity of the cold agglutinins present in the sera of their patients Johnsson (1945) referred to a patient suffering from an anæmia of unknown origin whose serum agglutinated samples of 80 bloods of all groups to a titre at 2 C of 32 000 and at 37 C of 2 000 The patient s own corpuscles were also agglutinated but were far less sensitive the titre at 37 C being 8 The nature of this remarkable agglutinin was not exactly established

Lubinski and Goldbloom (1946) described on the other hand auto antibodies which were active at 37 C and not potentiated by reduction in temperature and referred to several other previously reported cases in which agglutination had taken place at 37 C Young and Lawrence (1946) reported the presence of a non specific cold agglutinin which was still weakly active at 37 C in a patient of blood group A_2 in addition the patient s serum contained an α_1 agglutinin which was presumed to have developed as the result of previous transfusions Kuhns and Wagley (1949) also described the presence of two distinct antibodies in the serum of an acutely ill patient One antibody was a cold agglu tinin active against cells of all groups with a titre of 2 048 at 2 C but not active at all at 37 C the other was a warm antibody which agglutinated the patient s own erythrocytes as well as 63 % of a panel of normal erythrocytes apparently irrespective of their blood groups as far as they were known The antibody of Weiner and co workers (1953) and that of Case 12 also caused agglutination at 37 C (see p 204)

Warm Hæmolysins

Reports of hæmolysins in the sera of patients with acquired hæmolytic anæmia have always excited interest Such reports are rare however and some have been the cause of controversy The whole question was well reviewed by Dameshek and Schwartz (1940) and more recently by Dausset (1952) The early observa tions of Chauffard and Troisier (1908) and Chauffard and Vincent (1909) have already been referred to The latter paper is the more

specificity usually within the Rh system (see Chapter 9 p 233 for further details)

The antibodies may be divided on the basis of their laboratory behaviour into two main groups 'warm antibodies and "cold" antibodies (Dameshek 1951 Dacie and de Gruchy 1951 Bouroncle Dodd and Wright 1951 Young Miller and Christian 1951 Weiner Samwick Morrison and Loewe 1952 Baumgartner 1952 Dacie 1953) Warm antibodies are antibodies the activity of which is maximal at about 37 C cold antibodies on the other hand are antibodies the activity of which is markedly potentiated by cold usually they are only slightly active or completely inactive at 37 C Both types of antibody react *in vitro* as if they were incomplete antibodies (see p 29) Cold antibodies also act as complete' agglutinating antibodies and under certain circumstances bring about hæmolysis i e act as hæmolysins warm antibodies only occasionally act as agglutinating antibodies and rarely cause hæmolysis The types of antibodies capable of causing hæmolysis can also be shown to sensitize erythrocytes to phagocytosis (see p 14) The question whether the different phenomena of antibody action depend on *different components of antibody or are due to the same component* acting under different experimental conditions is dealt with in Chapter 9/

Literature on Serology

As described earlier in this chapter (p 165) the first observations which suggested that hæmolytic anæmia might be caused by the formation of auto antibodies were made in France in the first decade of the present century However it is only within the last ten years that real progress has been made and the abnormal antibodies studied in detail A complete review of all the recent work is out of the question however an attempt will be made to refer to those publications which seem to have contributed most to knowledge

Abnormal Cold and Warm Agglutinins

The early literature on acquired hæmolytic anæmia in which reference is made to autohæmagglutination or to the presence of cold agglutinins at pathological titres was reviewed by Dameshek and Schwartz (1940) and by Stats and Wasserman (1943) It is interesting to note though that as late as 1943 Stats and Wasserman concluded that the accumulated evidence favours the view that cold hemagglutination in these cases of hemolytic

Cold Hæmolysins

Antibodies capable of causing the hæmolysis of normal erythrocytes *in vitro* under certain conditions are regularly found in the sera of patients suffering from acquired hæmolytic anæmia of the cold antibody type associated with Raynaud s phenomena The main characteristics of this type of cold hæmolysin were described by Dacie (1950b) Prior to this publication only inconclusive reports were available such as those of Wyschegorodzewa (1926) Ernstene and Gardner (1935) and Salén (1935) and the very existence of cold hæmolysins (other than the Donath Landsteiner antibody) was doubted (Stats and Wasserman 1943)

Dacie (1950b) showed that sera containing cold antibodies at titres greater than about 1 000 at 2 C regularly caused lysis of normal erythrocytes if the serum corpuscle suspension was suitably acidified the optimum pH for lysis was found to be between 6 5 and 7 0 Hæmolysis readily took place at room temperature (20 C) but not at 37 C The lysins were thermostable and serum complement was required for the fixation of antibody as well as for lysis It was also shown that P N H erythrocytes were extremely sensitive to lysis by the antibodies Further examples of antibodies of this type were described by Dacie and de Gruchy (1951) and Ferriman and co workers (1951) and by Matthes and Schubothe (1951) Marcolongo (1953) van Loghem and co workers (1953) and Schubothe (1953) A more detailed description of the behaviour of the antibodies is given in Chapter 9 (p 2 0)

Incomplete Antibodies

Antiglobulin Reactions Boorman Dodd and Loutit (1946) and Loutit and Mollison (1946) were the first workers to demonstrate by means of the antiglobulin test the presence of abnormal incomplete antibodies in patients with acquired hæmolytic anæmia Their observations were soon confirmed by other workers throughout the world Denys and van den Broucke (1947) for instance reported positive direct tests in two patients one of whom was an infant and mentioned positive results in four others They also demonstrated the presence of free antibody in their patients sera and made interesting observations on the varying sensitivity of normal cells to the antibodies (see p 232) Sturgeon (1947) reported positive direct and indirect tests with the erythrocytes and serum of three patients He showed that the antibody could be eluted off washed erythrocytes by incubating saline suspensions of the cells at 37 C or at 56 C

Gardner s (1949) observations were also of importance He found that at a pH of 6 5 to 6 7 normal corpuscles or the patient s own corpuscles were agglutinated by the sera of thirteen out of 15 patients

convincing The patient was acutely ill and had hæmoglobinuria During the acute phase of his illness an autolysin and isolysin were demonstrated in his serum The lysin was most active at 37 C it was no longer found in the patient s serum on his recovery

Dausset (1952) listed eleven additional reports dealing with hæmolysins and hæmolytic anæmia which were published in France within a few years of Chauffard s papers None of the latter descriptions appears however to be as convincing as that of Chauffard and Vincent and nothing decisively new was discovered It was not in fact until 1938 that the role of hæmolysins in acute hæmolytic anæmia with hæmoglobinuria was re emphasized by Dameshek and Schwartz (1938a) Their report dealt with three patients in the first patient isohæmolysis but not autohæmolysis was demonstrated in the second only autohæmolysis the antibody of the third patient however hæmolysed the patient s corpuscles *in vitro* as well as normal corpuscles Like Chauffard Dameshek and Schwartz showed that the antibodies were thermostable and needed complement for lysis although they would fix antibody in the absence of thermolabile components of complement Dameshek and Schwartz also reported that the lysins they studied were active both at 18 C and at 37 C and were inactivated by the addition of normal human serum Another example of an abnormal lysin was reported by Farrar Burnet and Steigman (1940) The activity of this lysin was also said to be inhibited by normal serum

Effect of pH In 1944 David and Minot described the presence of an abnormal hæmolysin in the serum of an infant acutely ill with hæmolytic anæmia Normal erythrocytes as well as those of the patient were lysed *in vitro* and it was noted that the amount of lysis was increased by the addition to the serum of a one twentieth volume of N/3 hydrochloric acid

Dacie (1949) described in detail the presence of a hæmolysin in the serum of a girl acutely ill with an acquired hæmolytic anæmia and showed that the lysin s activity was markedly influenced by *p*H it was barely active in unacidified serum but strongly active at *p*H 6 8 to 7 0 Further details of this antibody are given in Chapter 9 Gardner and Harris (1950) also recorded briefly the demonstration in three patients of hæmolysins active at an acid *p*H

Dacie and de Gruchy (1951) reported five additional examples of hæmolysins apparently of the warm variety which they detected in the sera of patients suffering from idiopathic acquired hæmolytic anæmia These lysins however did not convincingly cause the lysis of normal corpuscles even at an acid *p*H normal corpuscles when trypsinized or paroxysmal nocturnal hæmoglobinuria (P N H) erythrocytes however were hæmolysed to quite high titres

nated in dilutions of autogenous serum in albumin in 17 out of 18 cases the titres ranging from 2 to 256 The majority of the antibodies were warm ones Lower titres were obtained using normal corpuscles The fact that the patients erythrocytes had already adsorbed antibody *in vivo* as shown by positive antiglobulin tests probably explains why the patients own corpuscles were agglutinated to higher titres than were the normal corpuscles (see p 23)

Use of Enzyme treated Erythrocytes Trypsinized erythrocytes (Morton and Pickles 1947 1951) have also been used to demonstrate the incomplete antibodies of acquired hæmolytic anæmia (Wheeler Luhby and Scholl 1950 Dacie and de Gruchy 1951 Wright Dodd Bouroncle Doan and Zollinger 1951 Bouroncle Dodd and Wright 1951 Foster and Hutt 1953 etc)

Dausset and Vidal (1951) reported observations on eight patients suffering from idiopathic acquired hæmolytic anæmia A variety of techniques was used including the direct and indirect antiglobulin methods auto agglutination in plasma albumin and antibody titration using normal corpuscles in plasma albumin and trypsinized corpuscles in saline dilutions of the patient s serum It was concluded that the strongest direct (corpuscular) reactions were associated with the most active hæmolysis *in vivo* and that the use of trypsinized corpuscles was the most delicate method of detecting antibodies in patients sera

Rosenthal Dameshek and Burkhart (1951) used trypsinized corpuscles at three temperatures 37 C 22 C and 3 C and compared the results with those obtained by titrating the antibodies using normal erythrocytes in saline and in albumin at the three different temperatures Whilst the results using the trypsinized corpuscles and those obtained with normal erythrocytes in albumin were of the same order Rosenthal and his colleagues thought that the trypsinized cells were less strongly agglutinated at 37 C than were the normal (not trypsinized) cells in albumin at 22 C the intensity of agglutination was about the same by the two methods but at 3 C the trypsinized cells were much more strongly agglutinated than were the normal cells in albumin

Individual Differences in Antibody Action Dacie and de Gruchy (1951) using both the antiglobulin method and trypsinized corpuscles published detailed findings on a relatively large number of patients They stressed the subtle differences in the behaviour of the antibodies of different patients and how more than one type of antibody might be present at the same time Their results are included in the personal observations referred to later in this chapter (p 190) Rosenthal Komninos and Dameshek (1953) also described a patient in whose serum several different antibodies appeared to be present

Serology of Acute Hæmolytic Anæmia (Lederer Type)

Until recently very little had been done to investigate cases of Lederer s anæmia from the serological point of view Now it is known that evidence of auto immunization may often be found

When the pH was raised to 8 0 agglutination was abolished but not sensitization to antiglobulin serum In only three instances did sensitization result when normal cells were exposed solely to the patients unacidified sera

Evans and Duane (1949) described eleven patients with acquired hæmolytic anæmia (one associated with chronic lymphatic leukæmia) whose erythrocytes gave positive antiglobulin tests Using dilutions of antiglobulin serum a fairly consistent correlation was found between the intensity of the reaction and the activity of the disease They reported however that in one patient an increase in the degree of sensitization as judged by the antiglobulin reaction was not associated with an immediate recurrence of the hæmolytic anæmia

Kidd (1949) investigated six patients with acquired hæmolytic anæmia whose erythrocytes gave strongly positive direct antiglobulin tests He showed that potent eluates of the antibodies could be prepared by elution from erythrocyte stromata at a low pH

Young Miller and Christian (1951) discussed in detail the use of the antiglobulin reaction in the diagnosis of hæmolytic anæmia of the auto immune type and drew attention to the technical difficulties in carrying out the test quantitatively The relationship between the laboratory tests for sensitization and the clinical course of the disease was illustrated by two case histories

Prozones in the Antiglobulin Test Van Loghem Stallman and Hart (1951) described the reactions of a cold antibody developed by a patient suffering from acquired hæmolytic anæmia associated with cirrhosis of the liver Van Loghem and his colleagues observed that when the patient s sensitized erythrocytes were suspended in concentrated highly potent antiglobulin serum agglutination was maximal They contrasted this with the inhibition of agglutination (prozone) which developed when the same antiglobulin serum was used in the same concentration to agglutinate the sensitized erythrocytes of three patients with idiopathic acquired hæmolytic anæmia (type Loutit) and corpuscles sensitized by anti D respectively

Neutralization of Antiglobulin Serum by γ Globulin Dacie (1951) concluded that whereas the warm antibodies of acquired hæmolytic anæmia were probably γ globulins the cold antibodies might not be γ globulins It was found that the agglutination of sensitized erythrocytes could be affected in different ways when human γ globulin was used to neutralize or partially neutralize the antibodies in the rabbit serum used in the antiglobulin test Whilst the agglutination of erythrocytes sensitized with the warm antibodies of acquired hæmolytic anæmia was abolished by the addition of small amounts of γ globulin to the antiglobulin serum much larger amounts of γ globulin were needed to inhibit the agglutination of erythrocytes sensitized by cold antibodies Crawford and Mollison (1951) extended this work and showed by absorption experiments that the warm and cold antibodies of acquired hæmolytic anæmia reacted with different components of the antiglobulin serum Dacie (1953) summarized the differences between cold and warm antibodies with particular reference to the antiglobulin reaction (see also p 235)

Titration in Albumin Dameshek (1951) reported the results of quantitative antibody titrations using a serum albumin technique (Neber and Dameshek 1947) The patients corpuscles were aggluti

present in every case irrespective of the presence of non specific cold antibodies

Minor degrees of macroscopic agglutination developed when the undiluted oxalated or heparinized whole blood of many of the patients was incubated at 37 C Microscopically the agglutination in most cases seemed to be at least in part due to marked rouleaux development—in some of the patients a pathologically raised serum globulin concentration was demonstrated In only one patient was the presence of an antibody

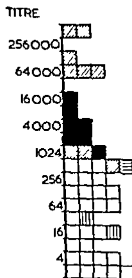


FIG 67 Cold agglutinin titres at 2° C obtained with the sera of 48 patients suffering from acquired hæmolytic anæmia of the auto-immune type Symbol as in Fig 6

capable of agglutinating saline suspensions of normal corpuscles at 37 C unequivocally demonstrated This patient (Case 12) was in an extremely acute hæmolytic pha e at the time the serum was obtained The antibody had an unusual specificity (see p 204)

Direct Antiglobulin (Coombs) Test This was positive in each case In several instances strongly positive reactions persisted in patients in whom good clinical remissions had been brought about by splenectomy (e g Case 9) The strengths of the reactions in different concentrations of antiglobulin serum and the effect on the reactions of the addition of γ globulin to the antiglobulin serum are considered on p 23. In most instances the antibody appeared to be a γ globulin

when sought for carefully Indications that this might be so can also be found in the older literature

In Chauffard and Vincent s (1909) case an abnormal hæmolysin was probably present and autoagglutination was noted in a few instances e g by Patterson and Stewart Smith (1936) by Giordano and Blum (1937 Case 2—probably a cold antibody) and by Greenwald (1938 Case 2)

More recently Hargraves Herrell and Pearman (1941) reported that erythrophagocytosis was a striking feature of the peripheral blood films of a patient in a severe hæmolytic episode The same phenomenon was observed by Landolt (1946) and Gasser and Hollander (1951) in fatal cases Microspherocytosis was conspicuous in both Landolt s and Gasser and Hollander s patients—a phenomenon not usually reported in typical Lederer s anæmia Warm auto antibodies were definitely present in Gasser and Hollander s case

Millichap (1952) described five further examples of acute hæmolytic anæmia in children Four patients recovered quickly (all were trans fused) the fifth child died hæmolysis persisting for three weeks and splenectomy bringing no benefit Of the four patients who recovered quickly the direct antiglobulin test was reported as positive in two the blood of both these patients underwent auto agglutination and their sera contained non specific antibodies acting upon normal corpuscles of the same blood group

Another recent series is that of Rose and Nabarro (1953) Their report dealt with four children all admitted to hospital in Leeds within a comparatively short time One patient recovered quickly after one transfusion had been given the other three recovered more slowly transfusion producing no more than temporary benefit Splenectomy carried out on two of them was not beneficial All three however responded to cortisone and A C T H therapy—in one patient this had to be kept up for 30 weeks The direct antiglobulin tests were positive in the latter three cases and non specific warm antibodies were detected in the patients sera

Personal Observations

The author has carried out serological studies in 30 patients suffering from idiopathic acquired hæmolytic anæmia of the warm antibody type and in nine patients with anæmia of the cold antibody type Some of the data obtained has already been published (Dacie and de Gruchy 1951 Ferriman *et al* 1951 Dacie 1953) The whole series will now be briefly reviewed Summarized data are given in Tables 7 and 8

Warm Antibodies (30 patients Table 7)

Cold Agglutinin Titre (at 2 C) This was within the normal range (i e 64 or less) except in ten patients in whom the titres ranged between 128 and 512 (Fig 67) Warm antibodies were

Indirect Antiglobulin Test Normal erythrocytes were sensitized in the patient's serum at 37 C and then tested for adsorbed antibody as described on p 487 Antibodies were detected by this method in the serum of 14 out of 21 patients in whom there was clinical and hæmatological evidence of active hæmolysis The test was however positive in only one out of seven patients in spontaneous remissions or in remissions induced by splenectomy The exact test for the comparison of two proportions indicates a significant difference (at the 5% level) between the two proportions It seems therefore that the presence of free antibody in the serum can be correlated with the existence of an active hæmolytic process In most instances the degree of sensitization was slightly increased by acidification of the serum to pH 6.5 to 6.8 as claimed by Gardner (1949) the effect being about the same as with anti D (Dacie 1953) It was interesting to note too that in three patients antibodies were present which sensitized cells to antiglobulin serum but did not agglutinate trypsinized cells (see p 240)

Agglutination and Hæmolysis of Trypsinized Normal Erythrocytes Trypsinized corpuscles were agglutinated at 37 C by the serum of 18 out of 23 patients in whom there was evidence for active hæmolysis and by the serum of five out of seven patients in remission

The correlation between the presence of antibody in the serum and active hæmolysis mentioned in the preceding paragraph applies to antibodies demonstrated by the indirect antiglobulin method and not to antibodies detected by means of trypsinized cells This was clearly shown in a patient who recovered from an acute hæmolytic episode and in whom the direct and indirect antiglobulin tests had become negative He was left nevertheless with an apparently non specific antibody in his serum which agglutinated trypsinized corpuscles to a titre of 1 024 The negative direct antiglobulin test demonstrated that the patient's unmodified erythrocytes were not capable of adsorbing the antibody

The sera of seven out of the 30 patients were found to be capable of bringing about lysis of trypsinized normal erythrocytes at 37 C The unacidified sera from four of these patients also hæmolysed paroxysmal nocturnal hæmoglobinuria (P N H) erythrocytes There was however no strict parallelism between the hæmolytic titres obtained with the two different types of abnormal cells The significance and nature of the hæmolytic antibody components is obscure only one of the sera hæmolysed normal (not trypsinized) corpuscles (see p 242)

The reactions of Cases 9 11 and 12 are contrasted in Table 8

TABLE 7 *Summarized Data on the Serological Findings in 30 Patients with Idiopathic Acquired Hæmolytic Anæmia of the Warm antibody Type The figures refer to the number of patients in whom the tests were positive or negative the figures in [ ] refer to the range of titres observed*

Number and clinical state of patients	Direct antiglobulin reaction		Cold agglutinin titre (°C)	Indirect antiglobulin reaction (3 °C)		Agglutination of trypsinized normal erythrocytes (3 °C)		Hæmolysis of trypsinized normal erythrocytes (3 °C)		Hæmolysis of P N H erythrocytes (3 °C)	
	+	−		+	−	+	−	+	−	+	−
23 Active hæmolysis	23	0	2–51[illegible]	14	7	18 [4–1 024]	5	7 [2–256]	16	4 [16–64]	19
In remission	7	0	2–2[illegible]	1	6	5 [2–1 024]	2	0	7	0	7

The sera of two patients (Case 13 and one other) which hæmolysed trypsinized normal erythrocytes at 37° C to quite high titres also hæmolysed P N H erythrocytes at 37 C The hæmolytic components in these sera behaved as warm antibodies and appeared to be distinct from the cold antibodies also present in the sera at moderately high concentrations They also appeared to be different from the hæmolytic antibodies present in the sera of the other cases of the cold antibody group the activity of which was markedly augmented by fall in temperature The contrasted reactions of Cases 13 and 14 are illustrated in Table 8 (p 203)

Other Serological Findings

Specific Immune iso antibodies Abnormal iso antibodies are not uncommonly found in the sera of patients who have received transfusions Anti E was identified in the sera of five patients of the author s series and anti E and anti c in a further patient

Hyperglobulinæmia The total plasma globulin concentration is not infrequently raised in acquired hæmolytic anæmia (Kracke and Hoffman 1943 Fisher 1947) and abnormal electrophoretic patterns and precipitation tests may be observed (e g Case 13 see also Young and Miller 1953b)

Serum Complement Gardner and Harris (1950) Dacie and de Gruchy (1951) and van Loghem and co workers (1952) have reported low levels of serum complement in several patients suffering from acquired hæmolytic anæmia Definitely abnormal levels have been found in four patients of the present series (Cases 13 and 14 and two others) It is perhaps significant that all four patients belonged to the cold antibody group

False positive Reactions for Syphilis Positive Wassermann and Kahn tests have been reported from time to time in patients with acquired hæmolytic anæmia which seem unlikely to be due to syphilis (Rosenthal and Corten 1937 Kracke and Hoffman 1943 Lubinski and Goldbloom 1946 Rubinstein 1948 Kracke and Riser 1949 Rosenthal Komninos and Dameshek 1953) In one patient although the Kahn and Wassermann reactions carried out on the patient s serum were negative an eluate made from the patient s erythrocytes reacted with the Kahn antigen (Gatman and Hamilton 1949) Both tests were positive in two patients of the present series In Case 13 the strength of the reactions gradually diminished and 3 years after splenectomy they were no longer positive (see p 207)

Cold Antibodies (9 Patients)

Direct Antiglobulin (Coombs) Test The tests were positive in each case the reactions on the whole being less strong than those produced by warm antibodies Agglutination took place best when the strongest concentrations of antiglobulin serum were used (see p 238)

Cold Agglutinin Titre The cold agglutinin titres using normal corpuscles ranged between 512 and 500 000 at 2 C (Fig 67) at higher temperatures the intensity of the agglutination and the titre of the serum was markedly reduced complete reversal of agglutination taking place in each case at temperatures between 28 and 32 C (Fig 76 p 249)

Indirect Antiglobulin Test Normal erythrocytes suspended at 20 C in the patient s acidified (*p*H 6 5) serum were strongly sensitized in each case in unacidified serum the reaction was usually less intense At temperatures above 20 C the degree of sensitization was less marked but in five out of nine cases the reactions were still weakly positive with cells sensitized at 37 C in acidified serum

Hæmolysis of Normal Erythrocytes The sera containing cold antibodies in very high concentrations rapidly hæmolysed normal corpuscles at 20 C if the *p*H of the serum cell suspension was adjusted to between 6 5 and 7 0 (see also p 250) In five patients the upper thermal limit for hæmolysis was approximately 30 C in the sixth patient (Case 14) a trace of lysis occurred at 37 C

The three sera containing cold antibodies at only moderately high titres (512–1 024) produced at the most only a trace of lysis of normal corpuscles

Agglutination and Hæmolysis of Trypsinized Normal Erythrocytes and P N H Erythrocytes Trypsinized erythrocytes were agglutinated at 2 to 20 C to very high titres by the sera of these patients however in most instances agglutination did not persist at 37 C At temperatures below 37 C lysis occurred in association with agglutination when the titrations were carried out in normal human serum in five patients some lysis developed at 37 C

P N H corpuscles were agglutinated by these sera to about the same titres as were normal erythrocytes when the sera were diluted in unacidified normal serum instead of in saline the P N H corpuscles were hæmolysed to about the same titres as they were agglutinated In six cases lysis took place at 37 C

of the warm antibody type Recovery was spontaneous and the patient has now been well for more than five years The direct anti globulin reaction however has remained weakly positive

Case Report Idiopathic Acquired Hæmolytic Anæmia (Warm antibody Type) Sustained Remission following Splenectomy

Case 9 The patient (S H) was a housewife who was admitted to hospital in October 1947 with a history that two years previously she had had an attack of painless jaundice associated with malaise dark urine weakness and anæmia The jaundice gradually faded but persisted for several months In March 1947 jaundice reappeared two weeks after an attack of influenza and never completely disappeared thereafter

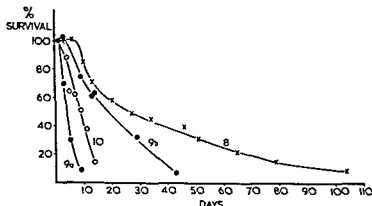


FIG 68 Survival of transfused normal erythrocytes in three patients suffering from idiopathic acquired hæmolytic anæmia (Cases 8 9 and 10) 9a = before splenectomy 9b = after splenectomy

The patient was one of two sisters who were probably identical twins their physical features were similar they had identically formed ear lobes similar palm prints identical thresholds for tasting phenyl thiocarbamide and identical ABO Rh MNS P *Lutheran Lewis* and *Duffy* blood groups The patient s twin sister was in good health and showed no signs of acquired hæmolytic anæmia

Physical Examination The patient was found to be an alert intelligent woman pale and visibly jaundiced A palpable spleen 5 cm below the costal margin was the only abnormal physical sign Her urine contained an excess of urobilin the fæces were normal in colour She remained under observation until October 28th 1947 when splenectomy was performed

Laboratory Findings Her erythrocyte count averaged 2 700 000 cells per c.mm with 10 3 g hæmoglobin per 100 ml and 12% reticulocytes the M C V was 103 c μ Examination of stained blood films revealed

Case Report Idiopathic Acquired Hæmolytic Anæmia (Warm antibody Type) Spontaneous Recovery

Case 8 The patient (L M) was a housewife aged 69 She was admitted to hospital complaining of tingling of the fingers for one year and also tinnitus deafness and giddiness For the last three months she had felt weak and had suffered from dyspnœa on exertion

Physical Examination She was found to be anæmic but not obviously jaundiced Otherwise the findings were essentially negative in particular the spleen was not palpable and no neurological signs were found which might have accounted for her symptoms Her urine contained an excess of urobilin but was otherwise normal

She was observed in hospital for three weeks during which time her condition was virtually unchanged

Laboratory Findings Her erythrocyte count averaged 2 300 000 cells per c mm and hæmoglobin 9 1 g per 100 ml The M C V was 106 c μ reticulocytes 8 3% plasma bilirubin 0 9 mg per 100 ml and the leucocyte count 8 000 cells per c mm with 50% neutrophils Examination of blood films showed slight macrocytosis anisocytosis and poikilocytosis some polychromasia and a mild degree of micro spherocytosis The erythrocyte osmotic fragility was slightly increased with a mall tail of fragile cells The bone marrow was hyperplastic the erythroid myeloid ratio being 1 0 8

Serology The patient s blood group was A cde/cde The direct anti globulin test was positive The Wassermann and Kahn tests were negative In September 1947 420 ml of her blood were removed by venesection and the patient was then transfused with two bottles of group O Rh negative blood followed by two bottles of group A Rh negative blood The survival of the group O blood was followed by the Ashby method using an anti A serum The rate of destruction was moderately increased the mean cell life being approximately 43 days 50% of the transfused corpuscles had been eliminated 27 days after transfusion Thereafter elimination proceeded at the normal rate and 10% of the transfused cells were still circulating 104 days after transfusion (Fig 68)

Further Progress The rise in hæmoglobin and erythrocyte count produced by the transfusion was maintained by the patient By the end of 1947 her blood count was virtually normal and the bilirubin level had fallen to 0 5 mg per 100 ml The direct antiglobulin test how ever was still positive

This patient has been examined subsequently at intervals for more than 5 years She has kept well and her blood count has been within the normal range throughout the whole period the erythrocyte count has varied between 4 100 000 and 5 000 000 cells per c mm and the hæmoglobin between 12 5 g and 15 7 g per 100 ml The reticulocyte count has ranged between 0 6 and 3 4% (average of 26 observations 2 0%) and the bilirubin concentration has kept between 0 2 mg and 0 7 mg per 100 ml The antiglobulin test has been weakly positive for the whole five years of follow up (see p 235) The results of tests for abnormal antibodies in the patient s serum have been consistently negative

Summary A case of chronic idiopathic acquired hæmolytic anæmia

at 37° C (Table 8) The antibody formed by the patient appears to be a non specific one

Summary A case of chronic idiopathic acquired hæmolytic anæmia of the warm antibody type There was a marked improvement in her clinical condition following splenectomy but laboratory tests suggested that excessive hæmolysis was still continuing although at a reduced rate The direct antiglobulin reaction remained positive for more than five years after splenectomy and its strength has been virtually unchanged The indirect antiglobulin reaction became negative after splenectomy although antibody in her serum could still be detected by the use of trypsinized corpuscles

Case Report Acquired Hæmolytic Anæmia (Warm antibody Type) A Chronic Case Responding Well to Cortisone

Case 10 The patient (F K) was a married woman aged 50 years She complained of jaundice and malaise which had been gradually increasing over a period of five months At the onset of the jaundice her urine was said to be dark and her stools pale The patient felt better after admission into hospital but her jaundice persisted and she became progressively more anæmic The stools were noted to be normal in colour In April 1953 she was transferred to Hammersmith Hospital through the courtesy of Dr N F Coghill

Physical Examination She was seen to be a pale and obviously jaundiced woman The liver could be felt 5 cm below the right costal margin it was firm and smooth to palpation and not tender The spleen was palpable 7 cm below the left costal margin Her urine contained excess urobilinogen but no bile

Laboratory Findings Her erythrocyte count on admission was 1 900 000 cells per c mm with 7 5 g of hæmoglobin per 100 ml The M C V averaged 109 c µ and the mean corpuscular hæmoglobin concentration 36 5% The total leucocyte count averaged 5 000 per c mm and the reticulocyte count varied between 17% and 24% There were 200 000 platelets per c mm The serum bilirubin concentration was 5 8 mg per 100 ml Erythrocyte osmotic fragility was slightly increased lysis commenced in 0 55% NaCl M C F being 0 47% NaCl

Examination of stained blood films showed a moderate amount of anisocytosis and polychromasia with a tendency to microspherocytosis In addition a number of pear shaped poikilocytes were present Bone-marrow biopsy revealed active erythropoiesis

The serum protein concentration was 8 4 g per 100 ml with 3 2 g albumin and 5 2 g globulin per 100 ml Electrophoresis revealed a high concentration of γ globulin The thymol turbidity was 22 units colloidal gold 5 units and alkaline phosphatase 24 units

Serology Her blood group was A cde/cde MN The direct antiglobulin reaction was positive the reaction behaving as if the antibody were a γ globulin Eluates were prepared from the patient s corpuscles and it was found that the antibody was of the warm type and that it was non specific Non specific antibodies were also present in her serum in low concentrations trypsinized normal corpuscles were agglutinated at 37° C but the indirect antiglobulin reaction was negative The cold agglutinin titre was 4

Liver Biopsy Liver biopsy was carried out twice On the first

slight anisocytosis and poikilocytosis with considerable polychromasia the erythrocytes were orthochromic and slightly macrocytic No *spherocytes were seen* There were 7 000 leucocytes per c mm with 75% neutrophils and 180 000 platelets per c mm The plasma bilirubin was 2 0 mg per 100 ml Erythrocyte osmotic fragility was normal The bone marrow was hyperplastic with an erythroid myeloid ratio of 1 1

Effect of Blood Transfusion She was given a transfusion of packed normal group O corpuscles When about 200 ml had been given the patient felt cold and faint and the transfusion was stopped she was *however none the worse subsequently* The elimination of the trans fused cells was followed by the Ashby method using an anti A serum Their survival was found to be markedly impaired the mean cell life of the transfused cells being approximately 5 days (Fig 68)

Serology (Table 8) The patient s blood group was A Cde/cDE The direct antiglobulin test was strongly positive The reaction was inhibited in strong concentrations of antiglobulin serum and also by the addition to the antiglobulin serum of very small concentrations of *γ globulin* The indirect antiglobulin reaction was positive using normal group O corpuscles sensitized in the patient s serum at 37 C the reaction was slightly enhanced by acidification of the patient s serum The serum also agglutinated at 37 C but did not hæmolyse trypsinized *normal erythrocytes* P N H erythrocytes were not hæmolysed *The* cold agglutinin titre was within the normal range and her blood did not undergo auto agglutination at room temperature The Wassermann and Kahn reactions were negative

Splenectomy The patient made a good *clinical recovery from* splenectomy Her jaundice disappeared and one month later her erythrocyte count had risen to 4 300 000 cells per c mm her hæmo globin was 13 g per 100 ml and the reticulocyte count had fallen to 3 2% *The bilirubin concentration was 0 5 mg per 100 ml* The antiglobulin test was still strongly positive The survival of normal corpuscles transfused at the time of the splenectomy was far better than that of the normal blood transfused before splenectomy (mean *cell life 26 days as compared with the pre operative figure of* 5 days) (Fig 68)

The *spleen* weighed 300 g Macroscopically it was not remarkable Sections showed normal Malpighian bodies and an increased cellularity of the pulp due principally to reticulum cell hyperplasia The amount of blood present was not abnormally great There was a moderate amount of iron containing pigment but little evidence of erythro phagocytosis

Subsequent Progress The patient has been examined at intervals for more than five years since splenectomy She has remained well throughout this period Nevertheless she has tended to be slightly anæmic and the plasma bilirubin concentration and reticulocyte count have been slightly above the normal range The average figures for the years 1950 to 1955 inclusive were as follows erythrocytes 4 000 000 per c mm hæmoglobin 12 9 g per 100 ml reticulocytes 3 3% and plasma bilirubin 0 8 mg per 100 ml

The direct antiglobulin test has remained strongly positive throughout the period The indirect antiglobulin test *has* become *negative* however her serum still agglutinates trypsinized normal erythrocytes

serum or in the intensity of the sensitization of her own corpuscles as judged by the direct antiglobulin test

Summary A case of chronic acquired hæmolytic anæmia of the warm antibody type The possibility of an underlying pathological process such as a reticulosis or sarcoidosis which was suggested by the first liver biopsy has neither been proved nor disproved A partial clinical and hæmatological remission followed treatment with cortisone

Case Report Idiopathic Acquired Hæmolytic Anæmia (Warm antibody Type) A Subacute Case Ultimately Proving Fatal

Case 11 The patient (J L) was a man aged 34 He was admitted to hospital giving a history of dyspnœa for the last three months and increasing palpitations and lassitude He also complained of a cough which had troubled him for about a month He had suffered from pertussis and pneumonia when a small child chorea when aged 13 and also from achalasia of the œsophagus for which he had undergone several operations the most recent being a cardioplasty in 1946

Physical Examination He was found to be anæmic and slightly jaundiced His spleen was palpable 8 cm below the costal margin In addition there were signs of mitral stenosis and aortic incompetence His urine contained urobilin but no bile pigment

There was no history of anæmia or jaundice in other members of the family

Laboratory Findings On admission on November 23rd 1948 his erythrocyte count was 3 400 000 cells per c mm hæmoglobin 10 5 g per 100 ml M C V 97 c μ reticulocytes 16% leucocytes 17 000 per c mm with 78% neutrophils and plasma bilirubin 2 1 mg per 100 ml Stained films showed well marked microspherocytosis and polychromasia (Fig 1 p 12) occasional normoblasts were also present Erythrocyte osmotic fragility was markedly increased lysis commencing in 0 ~5% saline The total serum protein concentration was 6 3 g per 100 ml with albumin 4 0 g and globulin 2 3 g per 100 ml His blood group was A Rh positive The Wassermann and Kahn reactions were negative

The blood counts and blood films of a sister and brother were normal

Further Progress Whilst under observation in hospital he rapidly became more anæmic By December 6th 1948 his erythrocyte count had fallen to 1 00 000 cells per c mm and the hæmoglobin concentration to 6 6 g per 100 ml The plasma bilirubin had risen to 3 ~ mg per 100 ml He was transfused with the packed cells from 2 pints of group O Rh positive blood and the fate of the normal corpuscles was followed by the Ashby method using an anti A serum The survival of the transfused cells was markedly impaired over 50% of the transfused blood having been eliminated by the 3rd day after transfusion It was shown too using the technique of differential agglutination that the normal erythrocytes were transformed into spherocytes after transfusion

The patient became more jaundiced after the transfusion and his spleen was found to have enlarged and to have become tender on palpation On December 9th 10th and 11th he was given further transfusions of 1 pint of packed cells but with little or no clinical or

occasion the presence of an irregularly distributed round cell infiltration and some collagen formation suggested an inflammatory process One epithelial cell focus with Langhans type giant cells was found A second biopsy however revealed only normal liver tissue

Further Progress On April 20th 1953 the patient was given a transfusion of two pints of group AN Rh negative blood and the fate of this blood was followed by the Ashby method using an anti M serum Its survival was markedly impaired (Fig 68) the mean cell life being approximately 8 days

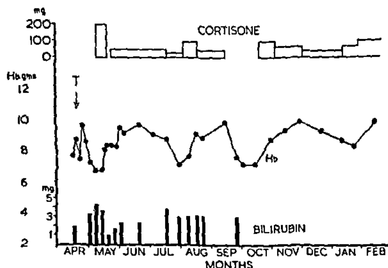


FIG 69 Hæmatological changes during the administration of cortisone to a patient suffering from idiopathic acquired hæmolytic anæmia of the warm antibody type (Case 10) T = transfusion

On May 8th 1953 the patient was given cortisone orally at the rate of 200 mg per day This was stopped after eight days because of symptoms of mental disorientation Treatment with smaller doses (50 mg daily) was recommenced after a pause of three days The effect on her blood was striking and almost immediate There was a rise in hæmoglobin and a fall in serum bilirubin concentration and in the reticulocyte count

Treatment with cortisone has now been kept up for 10 months with one short intermission the aim being to maintain a hæmoglobin concentration of 10 g per 100 ml with the smallest possible dose of cortisone A daily dose of 50 mg was found to be insufficient to keep her hæmoglobin and erythrocyte count in equilibrium but 100 mg a day proved to be more than enough for this purpose (see Fig 69) The patient has led an almost normal life whilst on treatment but has remained slightly jaundiced However despite the clinical and hæmatological improvement the treatment with cortisone did not seem to produce any substantial difference in the antibody concentration in her

TABLE 8 *Serological Observations on Five Patients suffering from Idiopathic Acquired Haemolytic Anaemia. Cases 9, 11 and 12 were of the warm antibody type, and Cases 13 and 14 of the cold antibody type. The abnormal auto antibodies appeared to be "non specific" in Cases 9, 11, 13 and 14; that of Case 12 was a mixture of anti C and anti e*

Case number and clinical state of patients	Direct antiglobulin reaction	Agglutination of normal erythrocytes (Titre)		Indirect antiglobulin reaction		Haemolysis of normal erythrocytes pH 6.5 (Titre)		Haemolysis of P.N.H. erythrocytes pH 8.0 (Titre)		Agglutination of trypsinized normal erythrocytes (Titre)	Haemolysis of trypsinized normal erythrocytes pH 8.0 (Titre)
		16° C	37° C	37° C	20° C	37° C	20° C	37° C	20° C	37° C	37° C
9 (In remission)	+++	4	—	—	—	—	—	—	—	8	—
11 (Acute haemolysis)	+++	256	—	++	++	—	—	—	—	64	—
12 (Hyperacute haemolysis)	+++	512	1,024	+++	+++	—	—	—	—	1,024	—
13 (Active haemolysis)	+	1,024	—	+	++	—	trace	512	512	32	128
14 (Active haemolysis)	+	512,000	—	+	+++	trace	++	4	16,000	—	—

+ denotes a positive reaction, ++ and +++ denote strongly positive reactions, — denotes a negative reaction

hæmatological benefit On December 13th he was much worse His urine was deep red due to hæmoglobinuria and the plasma bilirubin concentration had risen to 5 0 mg per 100 ml Further transfusion was of no benefit his urine became black ' with hæmoglobin and he died later that day

As his disease progressed spherocytosis increased markedly in intensity and the erythrocyte osmotic fragility became still more abnormal Seven per cent of his corpuscles were siderocytes The leucocyte count rose terminally to 28 000 cells per c mm

The patient s whole blood whether defibrinated or heparinized underwent a relatively rapid spontaneous lysis at 37 C On December 6th definite lysis was appreciable after 6 hours incubation and this was intense (21%) after 24 hours at 37 C On December 9th lysis commenced after about 3 hours incubation His bone marrow was markedly hyperplastic Erythropoiesis was normoblastic on December 7th the erythroid myeloid ratio was 3 1

Serology (Table 8) The direct antiglobulin test was strongly positive Serum obtained on December 6th 1948 was found to contain an antibody which agglutinated at 37 C trypsinized normal erythrocytes in saline and normal erythrocytes in albumin to titres of 32 and 16 respectively Neither normal corpuscles when trypsinized nor P.N H erythrocytes underwent hæmolysis A cold agglutinin was present which agglutinated normal corpuscles to a titre of 256 at 2 C and 4 at 18 C The warm antibody in the patient s serum behaved as if it were an antibody of γ globulin type The indirect antiglobulin reaction was positive when sensitizations were carried out at 37 C the reaction being enhanced by acidifying the serum corpuscle suspension to *p*H 6 5 to 7 0 The reaction was not inhibited by previously inactivating the serum at 56 C for 30 minutes The antibody appeared to be a non specific one

Postmortem Examination The *spleen* weighed 850 g microscopically it was grossly congested with blood in some places infarction had occurred There was in addition a generalized reticulum cell hyperplasia The *liver* showed centrilobular congestion small areas of necrosis and some foci of extramedullary hæmopoiesis In the kidneys there were signs of early siderosis of the convoluted tubules and some cast formation without however evidence of pigment nephrosis

Other findings included marked dilatation of the œsophagus old rheumatic endocarditis and lipoid pneumonia of the lungs probably due to inhalation of liquid paraffin used in passing œsophageal bougies

Summary A case of idiopathic acquired hæmolytic anæmia of the warm antibody type which terminated in an acute hæmolytic episode causing the death of the patient Blood transfusion was of no benefit

Case Report Idiopathic Acquired Hæmolytic Anæmia Supervening on Thrombocytopenic Purpura Death as the Result of a Hyperacute Hæmolytic Episode

Case 12 The patient (R E) was a woman aged 43 years In 1939 14 years before her final illness she noticed purpura for the first time In 1942 she again complained of purpura bruising of her legs and of several episodes of severe nose bleeding She was found to have

As it was not possible to find any blood which was compatible *in vitro* with the patient s serum the blood chosen for the transfusion was that which appeared to be least incompatible This blood was found however by Dr P L Mollison using the Ashby method to be destroyed as fast as it was transfused The patient died two days after admission into hospital (In retrospect the patient should have been transfused with cDE/cDE blood which was in fact compatible *in vitro* Unfortunately the most unusual and unexpected specificity of the patient s antibody was not discovered until after her death)

Summary A case of fatal hyperacute acquired hæmolytic anæmia in a patient previously splenectomized for thrombocytopenic purpura The auto antibody formed was most unusual in its specificity It was found to be a mixture of two Rh antibodies anti-e and anti C no abnormal strictly non specific antibodies were identified

Case Report Idiopathic Acquired Hæmolytic Anæmia (Cold antibody Type) Sustained Remission Following Splenectomy

Case 13 The patient (L R) was a housewife aged 54 She was admitted into hospital in August 1950 on account of a variety of complaints including intermittent diarrhœa night sweats and migrainous attacks accompanied by vomiting from all of which she had suffered for years In addition she had noticed for the previous year or so that she became slightly yellow from time to time and also that she had a swelling in the left side of her abdomen

Physical Examination This revealed pallor of the mucous membranes and a just perceptible tinge of jaundice considerable enlargement of the spleen which reached a level slightly below the umbilicus and slight enlargement of the liver The superficial lymph nodes were not enlarged to palpation and there was no purpura of the skin or mucous membranes The urine contained an excess of urobilin but was otherwise normal The fæces were normal in colour

She remained under observation for about 4 months during which time there was little change in her clinical state or laboratory findings At the end of this time splenectomy was carried out

Laboratory Findings (before splenectomy) The erythrocyte count averaged 2 500 000 cells per c mm hæmoglobin 10 3 g per 100 ml M C V 113 c µ reticulocytes 5 % leucocyte count 3 100 cells per c.mm with 50 % neutrophils platelet count 42 000 per c mm and bilirubin 1 3 mg per 100 ml

Her blood underwent autoagglutination at room temperature immediately after withdrawal but this was reversed on incubation at 37 C Stained smears showed a tendency to macrocytosis with slight anisocytosis and poikilocytosis and a moderate degree of polychromasia No abnormal leucocytes were seen Platelets were visible but were present in reduced numbers The erythrocyte osmotic fragility was within the normal range The patient s blood group was O M Rh positive

The Wassermann and Kahn reactions were strongly positive The total serum protein concentration was 6 9 g per 100 ml with albumin 3 5 g and globulin 3 4 g per 100 ml The thymol turbidity was 13 units and alkaline phosphatase 12 0 units the colloidal gold test was

thrombocytopenia Splenectomy was carried out and following this her platelet count rose to above the normal level She remained well for two years but in 1949 and again in 1950 purpura and thrombocytopenia reappeared She was treated by transfusion In 1950 she became jaundiced and hæmolytic anæmia was diagnosed the erythrocyte osmotic fragility was markedly increased and the direct antiglobulin test was positive Later her blood count became normal once more In 1951 she developed polyneuritis She recovered from this *after 4 weeks In 1952* there was a further short episode of purpura and thrombocytopenia Two days before admission into Hammersmith Hospital in January 1953 she became acutely ill with pallor jaundice lassitude and hæmoglobinuria

Physical Examination She was found to be jaundiced and very pale There was no purpura or other signs of a bleeding diathesis Her liver was palpable 5 cm below the right costal margin The superficial lymph nodes were not significantly enlarged Her urine was dark reddish brown in colour due to the presence of methæmoglobin *and hæmatin*

Laboratory Findings The patient s erythrocyte count on admission was 1 400 000 cells per c mm with 6 3 g hæmoglobin per 100 ml The M C V was 106 c μ and the M C H C 42% There were 20% reticulocytes The leucocyte count was 32 000 cells per c mm with 70% neutrophils and occasional myelocytes The platelet count was 220 000 per c mm The bleeding time clot retraction and coagulation time were all normal Examination of stained blood films showed auto agglutination and an extreme degree of spherocytosis (Fig 64) all the *erythrocytes except the reticulocytes being affected* Thirty normoblasts per 100 leucocytes were present A few examples of erythrophagocytosis by monocytes were found

Blood withdrawn from the patient was visibly agglutinated The auto agglutination did not disperse at 37 C There was obvious hæmoglobinæmia the hæmolysis rapidly became much more intense *in vitro* so much so that when her blood was allowed to clot at 37 C free hæmoglobin could be seen to diffuse out from the clot as it was retracting i e autohæmolysis was taking place within half an hour of the collection of the sample This rapid autohæmolysis took place whether or not the blood was oxalated heparinized or defibrinated Osmotic fragility was markedly increased 4% hæmolysis even taking place in 0 85% saline the M C F was 0 75% NaCl Spectroscopic examination of her serum showed the presence of a great deal of *methæmalbumin as well as oxyhæmoglobin*

Serology (Table 8) The patient s blood group was B Cde/cde The direct antiglobulin test was very strongly positive A warm auto agglutinin was present in the patient s serum in high titre (1 024) This was found subsequently to be capable of agglutinating normal and trypsinized corpuscles of all groups except those *of the Rh genotype* cDE/cDE Further investigation showed that the antibody was a mixture of anti e and anti C both capable of causing agglutination in saline dilutions (see p 233) No abnormal warm non specific antibodies were present The cold agglutinin titre using cDE/cDE corpuscles was within the normal range

Further Progress The patient was treated with A C T H given intravenously in the form of a saline drip and also by blood transfusion

subnormal after operation and the Wassermann and Kahn reactions also were at first strongly positive when performed however two years after splenectomy the Wassermann reaction was negative although the Kahn test was still positive Three years after splenectomy both tests were negative

Spleen The spleen weighed 1,375 g The surface was smooth the cut surface was a uniform deep red with visible Malpighian corpuscles

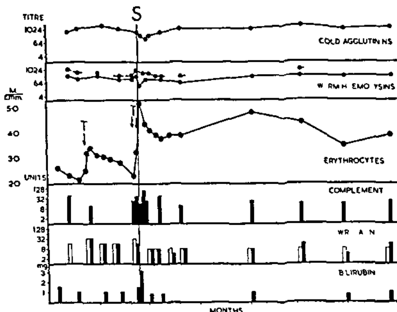


Fig 70 Hæmatological changes resulting from splenectomy in a patient suffering from idiopathic acquired hæmolytic anæmia of the cold antibody type (Case 1d) T = transfusion S = splenectomy ●- - -● = warm hæmolysin titre using P N H erythrocyte ●——● = warm hæmolysin titre using trypsinized erythrocytes ▮ = quantitative Kahn tests ▯ = quantitative Wassermann reaction

Histological sections showed a large amount of blood within the spleen pulp and also in dilated sinuses There was a moderate degree of hyperplasia of reticulum cells and some evidence of erythrophagocytosis by macrophages Blood from the splenic vein contained 3 mg bilirubin per 100 ml

Summary A case of chronic acquired hæmolytic anæmia of the cold auto antibody type In addition to the cold antibody a warm component of antibody acting upon trypsinized and P N H corpuscles was constantly present There was evidence of a considerable disturbance of protein formation and the serum gave false positive Wassermann and Kahn reactions

negative Paper strip electrophoresis showed a raised γ globulin content (23%) and a slightly low content of β globulin

Effect of Blood Transfusion The survival of transfused normal erythrocytes of group O N was studied by the Ashby method using anti M globulin (Lederle) The transfused erythrocytes were eliminated at an accelerated rate the mean cell life before splenectomy being 11 5 days (Figs 22 and 23 pp 35 and 36)

Serology (Table 8) The cold agglutinin titre of her serum was consistently in the region of 1 024 at 2 C 16 at 20 C and 4 at 25 C At *temperatures of 30 C or above normal erythrocytes were not* agglutinated However her serum agglutinated at 37 C and in the presence of fresh human serum hæmolysed trypsinized normal erythrocytes P N H erythrocytes were also hæmolysed at 37 C to a high titre

The direct antiglobulin (Coombs) test was consistently positive agglutination was most intense in the strongest concentrations of antiglobulin serum and the reaction was relatively insensitive to the addition of γ globulin to the antiglobulin serum The indirect antiglobulin test was positive using normal group O corpuscles when sensitizations were carried out at 37 C but *only if the serum was acidified* sensitization was completely inhibited if the patient s serum had been previously inactivated at 56 C The reactions were much stronger if the sensitizations were carried out at room temperature

The reactions outlined above are characteristic of a non specific cold antibody capable of sensitizing corpuscles at temperatures extending up to 37 C In addition it seemed that the patient s serum contained a separate warm antibody component capable of causing the hæmolysis of trypsinized and P N H erythrocytes at 37 C (see also p 243) The serum complement concentration was greatly reduced (10 to 32 units compared with a normal range of 70 to 150 units)

Splenectomy Splenectomy was carried out on November 24th 1950 The patient s subsequent progress is illustrated in Fig 70 She was transfused at the time of operation the survival of the transfused blood being followed by the Ashby method It was found that the rate of elimination of the transfused corpuscles was substantially less than before operation (Figs 22 and 23) Clinically the patient was greatly improved and her jaundice disappeared

The patient s progress has been followed for three years since splenectomy Her blood has been examined eight times during the last two years The results suggest that hæmolysis has continued but at a reduced rate The erythrocyte count has averaged 3 700 000 cells per c mm and hæmoglobin 13 1 g per 100 ml Her total leucocyte count has averaged 6 700 per c mm with 13% to 37% neutrophils and the platelet count 170 000 per c mm The reticulocyte count has ranged between 1 1 and 3 8% with an average of 2 7% and the plasma bilirubin has averaged 0 8 mg per 100 ml Her serum globulin has remained slightly raised and the thymol turbidity test has been constantly abnormal

Effect of Splenectomy on Serology No substantial changes in the concentration of antibodies resulted from splenectomy (Fig 70) In particular *the cold agglutinin and warm hæmolysin titres* have remained almost unaltered Similarly the direct antiglobulin test has been consistently positive The serum complement level remained

at 37 C perfectly clear unhæmolysed serum could be obtained Blood counting was easily accomplished if warmed blood was diluted in diluting fluid previously warmed at 37 C and a warmed counting chamber used

The direct antiglobulin test was positive even if precautions were taken to avoid cooling the blood after withdrawal The reaction was of the cold antibody type (see p 204) Cold agglutinins were present in her serum in very high concentrations Titration with normal group O corpuscles gave the following results 2 C, titre 512 000 13 C 32 000 17 C 8 000 20 C 512 25 C 32 30 C 4 At 20 C normal corpuscles were readily hæmolysed by her acidified serum (pH 6 5) a trace of lysis developed in unacidified serum at this temperature At 37 C no lysis of normal corpuscles took place in unacidified serum but there was a trace in acidified serum P.N H erythrocytes were rapidly hæmolysed to a titre of 16 000 at 20 C at 37 C a trace of lysis was produced by a 1 in 4 dilution of the patient s serum Trypsinized normal erythrocytes were very strongly agglutinated in the cold at 15 C they were hæmolysed to a titre of 1 024 no lysis took place at 37 C

The indirect antiglobulin test was strongly positive when sensitizations were carried out at room temperature particularly if the serum was acidified at 37 C the tests were much less strongly positive Indirect antiglobulin tests were consistently negative if the patient s serum had been previously inactivated by heating at 56 C for 30 minutes The above results are typical of an antibody of cold type present in high concentrations and with a high thermal amplitude

The patient s serum complement concentration was subnormal (15 units normal range 70 to 150 units) Complement fixation tests were carried out with the patient s serum and Influenza A and B Q fever and *Psittacosis* antigens and agglutination tests carried out using *Streptococcus* M G The results of all these tests were within the normal range

Clinical Progress The patient was successfully transfused on nine occasions between May and July 1951 without serious reactions The benefit derived from transfusion was however only transitory Two of the transfusions consisted of group O Rh positive blood the survival of which was followed by the Ashby method using an anti A serum the mean cell life was estimated to be approximately 5 days and 11 days respectively

The patient s clinical state remained unaltered between July 1951 and April 1953 The hæmatological findings likewise showed relatively little alteration the erythrocyte count varied between 2 500 000 and 3 400 000 cells per c mm with between 4 % and 16 % reticulocytes the leucocyte count varied between 1 800 and 8 000 cells per c mm with an average of 3 700 cells per c mm the serum bilirubin averaged 2 0 mg per 100 ml The serological findings were also substantially unchanged the direct antiglobulin test was constantly positive and the cold agglutinin titre using normal group O corpuscles remained within the range 128 000 to 512 000 at 2 C

At the beginning of April 1953 it was decided to give cortisone a trial at an initial dosage of 200 mg daily by mouth The response to this

[1] Data quoted through the courtesy of Dr P L Mollison

Splenectomy was followed by a good clinical response although antibody formation seemed hardly to be affected by the operation and slight anæmia persisted

Case Report Idiopathic Acquired Hæmolytic Anæmia (Cold antibody Type with Raynaud s Phenomena) Improvement on Cortisone

Case 14 The patient (L S) was an elderly woman She was first admitted to hospital in October 1950 when aged 75 years complaining that for the last three months she had suffered from weakness and lassitude There was no evidence of any relevant antecedent illness She also complained that her hands became cold blue and numb in cold weather Hæmolytic anæmia associated with cold agglutinin formation was diagnosed and she was treated by means of blood transfusions receiving 16 pints in all These transfusions resulted in only transient benefit In May 1951 she was transferred to Hammersmith Hospital through the courtesy of Dr L I M Castleden for further investigation

On examination in May 1951 she was found to be an elderly and rather wasted woman She was pale and definitely jaundiced Her spleen was enlarged its lower edge being palpable 5 cm below the left costal margin Her hands were usually purplish in hue and very cold they became a normal colour when placed in warm water The tip of her nose and ear lobes also became markedly cyanosed when exposed to cold X ray examination of her chest showed a mottled opacity in the upper zone of the left lung Her cardiovascular system was normal for her age Her urine contained an excess of urobilin but no bile She was afebrile

The patient was under observation in hospital for approximately two months during which time she received a course of chloramphenicol and aureomycin for her lung condition which was assumed to be of inflammatory origin without however altering its radiographic appearance significantly

Laboratory Findings On admission her erythrocyte count averaged 2 000 000 cells per c mm hæmoglobin 7 6 g per 100 ml M C V 109 c μ reticulocytes 14% total leucocytes 5 500 per c mm with 50% neutrophils platelets 125 000 per c mm and plasma bilirubin 2 5 mg per 100 ml Examination of stained peripheral blood films showed a moderate degree of anisocytosis with slight poikilocytosis and a marked degree of polychromasia Occasional spherocytes could be seen as well as an occasional normoblast Erythrocyte osmotic fragility was slightly increased lysis commenced in 0 525% NaCl the M C F being 0 465 % NaCl The total plasma protein concentration was 7 3 g per 100 ml with 3 8 g albumin and 3 5 g globulin per 100 ml Electrophoresis showed raised α_1 and β globulin concentrations the γ globulin concentration was normal The Wassermann and Kahn reactions were negative

Serology (Table 8) The patient s blood group was A Rh positive On withdrawal from the body her blood underwent rapid massive auto agglutination At temperatures above 30 C however this did not occur and if blood was delivered into containers previously warmed

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was quite dramatic and rapid (Fig 71) Within a week the erythrocyte count rose by more than 500 000 cells per c mm and the packed cell volume from 27% to 37% the patient s leucocyte count also rose from 2 500 to 7 000 cells per c mm

The later effect of cortisone therapy is also illustrated in Fig 71 The improvement in the patient s clinical condition and blood count was sustained on a daily dosage of 100 mg to 125 mg if the dose of

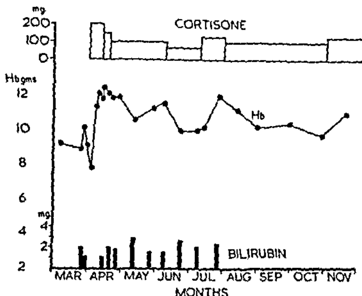


FIG 71 Hæmatological changes during the administration of cortisone to a patient suffering from idiopathic acquired hæmolytic anæmia of the cold antibody type (Case 14)

cortisone was reduced to less than 100 mg per day the blood count fell only to be regularly restored on increasing the dose once more The treatment with cortisone however did not seem to alter significantly the patient s cold agglutinin titre or the intensity of the direct antiglobulin test

Summary A case of chronic hæmolytic anæmia of the cold antibody type associated with Raynaud s phenomenon The patient s clinical state and laboratory findings have remained relatively unchanged for more than two and a half years Sustained benefit followed treatment with cortisone

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PATTERSON H W and STEWART SMITH G (1936) Acute hæmo lytic anæmia of Lederer in a child *Lancet* ii 1096
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CHAPTER 8

ACQUIRED HÆMOLYTIC ANÆMIA (AUTO ANTIBODY TYPE)

II HÆMOLYTIC ANÆMIA FOLLOWING OR ASSOCIATED WITH VIRUS INFECTIONS

ACUTE HÆMOLYTIC ANÆMIA FOLLOWING VIRUS PNEUMONIA

History The frequent development of cold antibodies in high concentrations by patients suffering from virus (primary atypical) pneumonia was first conclusively demonstrated by Peterson Ham and Finland (1943) Turner (1943) Horstmann and Tatlock (1943) and Turner Nisnewitz Jackson and Berney (1943) Isolated instances of an unusual degree of autohæmagglutination in patients suffering from respiratory infections however had been noticed before this Both Clough and Richter (1918) and Wheeler Gallagher and Stuart (1939) carried out detailed studies on their patients sera but the possible connection between the abnormal agglutinins and the preceding infection was not appreciated Clough and Richter finding cold agglutinins in the blood of a daughter of their patient in fact erroneously concluded that the abnormality might have been inherited

In 1943 Peterson Ham and Finland and Horstmann and Tatlock referred briefly to instances of acute hæmolytic anæmia amongst the patients of their series and two other possible examples were reported by Dameshek (1943) Further instances were described by Finland Peterson Allen Samper and Barnes (1945) by Ginsberg (1946) and by Colmers and Snaveley (1947) and more recently by Besterman and Brigden (1949) Neely Baria Smith and Stone (1951) Siegenthaler (1952) and Aaron (1952) etc Other possible examples can be found in the older literature e g the second patient described by Giordano and Blum (1937) as suffering from an acute hæmolytic anæmia of the Lederer type

The syndrome is undoubtedly less rare than the small number of published case reports suggests Dacie and de Gruchy (1951) for instance described the serological findings in four hitherto unpublished examples of the condition and two further cases are described on pp 271-275

WHITTLE C H LYELL A and GATMAN M (1947) Raynaud s phenomenon with paroxysmal hæmoglobinuria caused by cold hæmagglutination *Proc R Soc Med* 40 500
WIDAL F ABRAMI P and BRULÉ M (1908a) Les ictères d origine hemolytique *Arch Mal Coeur* 1 193
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YOUNG L E MILLER G and CHRISTIAN R M (1951) Clinical and laboratory observations on autoimmune hemolytic disease *Ann intern Med* 35 507
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the cold agglutinin titre in the patient (Case 15) whose history is described on p 221 is illustrated in Fig 72

The antibodies are potentially hæmolytic (Dacie 1949 Dacie and de Gruchy 1951) and normal erythrocytes were hæmolysed quite rapidly at room temperature (15–20° C) in the acidified serum of all of the seven patients the author has studied Trypsinized normal erythrocytes and more especially P N H corpuscles were hæmolysed at 15–20 C in high serum dilutions Similarly the indirect antiglobulin reaction carried out with normal corpuscles sensitized in patients sera at 15–20 C was strongly positive particularly if the sera were acidified (Table 9)

TABLE 9 *Serological Data on Two Patients suffering from Acute Hæmolytic Anæmia following Virus Pneumonia*

Case No	Direct antiglobulin reaction	Cold agglutinin titre (4° C)	Indirect antiglobulin reaction (pH 6 5)		Hæmolysis of normal erythrocytes (pH 6 5)		Hæmolysis of P N H erythrocytes (pH 8 0)		Hæmolysis of trypsinized normal erythrocytes (pH 8 0)	
			37° C	20 C	37° C	20° C	37° C	20 C	37 C	20° C
15	+	8 000	±	++	–	++	16	? 018	–	64
16	+	4 000	++	++	–	++	16	2 6	4	

++ denotes a strong reaction + denotes a moderately strong reaction and ± a weak reaction
– denotes no hæmolysis and no observation The figures indicate the cold agglutinin and hæmolysin titres respectively

The *direct antiglobulin test* was positive at the time of the hæmolytic episodes in all the cases the author has studied even if the blood was collected directly into saline previously warmed to 37 C This suggests that the antibodies have a high thermal amplitude which is confirmed by the fact that tests for antibodies in the patients sera are often positive if sensitive methods are used when the sensitizations are carried out strictly at 37 C For instance in three out of seven cases the indirect antiglobulin test was positive using normal erythrocytes sensitized in acidified serum at 37 C whilst five out of six (unacidified) sera caused lysis of P N H corpuscles at 37 C Normal erythrocytes were not lysed at 37 C but some lysis was produced at 30 C by the acidified serum of five out of six patients investigated The serum of one patient hæmolysed trypsinized normal erythrocytes at 37 C (see below)

Clinical Features

When hæmolysis occurs after virus pneumonia it does so usually towards the end of the second week or during the third week of the patient s illness The onset of hæmolysis is usually sudden The patient who may have already recovered from his respiratory infection becomes ill once more with increasing pallor and jaundice and prostration There may even be hæmoglobinuria (Dameshek 1943 Horstmann and Tatlock 1943 Neely *et al* 1951) The spleen often, but not invariably becomes palpable

In rare instances gangrene of the extremities has developed in the course of the illness Carey Wilson and Tamerin (1948) reported that the feet of their patient a woman aged 31 became gangrenous and Rønner Jessen (1950) mentioned that gangrene of the finger tips developed in an elderly man aged 75 In these patients the gangrene was presumably due to thrombosis following intense intravascular autohæmagglutination

Laboratory Observations

Autohæmagglutination *in vitro* at room temperature is an invariable and striking finding Anæmia is often severe and rapidly increases erythrocyte counts as low as 1 000 000 cells per c mm having been recorded The total leucocyte count may be markedly raised (Dameshek 1943 Horstmann and Tatlock 1943 Aaron 1952) counts exceeding 40 000 cells per c mm being not uncommon Most of the cells are neutrophils but myelocytes may be present in small numbers Peripheral blood films show in addition to autohæmagglutination a variable degree of polychromasia depending upon the stage of the disease and as a rule moderate to marked spherocytosis Erythrocyte osmotic fragility is usually increased to a moderate degree

The serum bilirubin concentration is usually raised to between 1 and 3 mg per 100 ml The plasma hæmoglobin level is almost invariably above normal and Schumm s test is positive in most cases Aaron (1952) reported a temporary rise in serum globulin concentration to 5 6 g per 100 ml in one case

Serology In all the cases so far recorded the abnormal antibodies have been of the cold variety The cold agglutinin titres at the time of onset of hæmolysis have been reported as being within the range 512 to 32 000 at 2–4 C and it is interesting to note that the highest concentration of antibodies (titre 32 000) was found in the serum of the patient whose feet became gan grenous (Carey Wilson and Tamerin 1948) The rise and fall in

Treatment

The patients should be kept warm in bed. This is particularly important as chilling is likely to cause an increase in hæmolysis. This is illustrated by the case report of Colmers and Snaveley (1947) whose patient was sponged with iced alcohol in an attempt to treat her hyperpyrexia. On the following morning she was moribund.

Transfusion should be reserved for the most severely anæmic patients. It is probably advisable to warm the transfused blood to body temperature before administration. (The selection and cross matching of blood for transfusion in cases of acquired hæmolytic anæmia is considered in Chapter 12.)

There seems no reason to contemplate splenectomy in acute hæmolytic anæmia following virus pneumonia, as the episodes of hæmolysis are normally of short duration. The same applies to treatment with cortisone or A.C.T.H. However, if hæmolysis is unusually severe, or if the diagnosis is in doubt, these drugs should be used (see p. 3-3).

Case Report. Acute Hæmolytic Anæmia probably following Virus Pneumonia. Spontaneous Recovery

Case 15. The patient[1] (M. A.) was a man aged 35. He was admitted to hospital in July 1948 with a history that he had felt unwell for the previous two days. He complained of sore throat, aching in the back and limbs, and headache. On admission he was found to be slightly delirious and during the afternoon he had a rigor; his temperature reached 105° F. Nothing abnormal was found on physical examination except for indications of consolidation at the base of the right lung. Later he coughed up some "rusty" mucopurulent sputum. His urine contained a trace of albumin but was otherwise normal. A radiograph of his chest revealed consolidation affecting the right middle lobe.

Laboratory Investigations. On admission there were no striking abnormalities in the blood picture; the hæmoglobin concentration was 14.5 g. per 100 ml. and there were 8,000 leucocytes per c.mm. with 84% neutrophils. His cerebro-spinal fluid contained 65 mg. protein per 100 ml. but was otherwise normal. A blood culture was sterile. Sputum culture yielded a moderate growth, mainly of *Strept. viridans*. The cold agglutinin titre was 4.

Further Progress. Pneumonia was diagnosed and between July 8th and 13th he was given 34 g. of sulphamethazine. Although there was no dramatic response to this treatment, the pyrexia slowly subsided and the patient felt better. On July 15th a course of intramuscular penicillin

[1] A more detailed clinical history of this patient was given by Besterman and Brigden (1949); the serological findings were referred to briefly by Dacie (1949).

Although the reactions of the antibodies the author has investigated have in general been similar there have been interesting minor variations For instance although the incomplete antibody of Case 16 behaved like a cold antibody in requiring fresh serum for sensitization it nevertheless sensitized normal corpuscles as intensely at 37 C as it did at 2 C or 20 C The serum of Case 15 of Dacie and de Gruchy (1951) was also unusual At the time of this patient s hæmolytic crisis her serum contained a factor which quickly hæmolysed trypsinized normal erythrocytes the optimum temperature for this factor was approximately 37 C the hæmolytic titre being lower when sensitization was carried out at 20 C The warm hæmolytic factor disappeared from the patient s serum during convalescence The other sera the author has investigated behaved in exactly the opposite and more usual way lysis of trypsinized corpuscles taking place at 20 C but not at 37 C

The individual variations and the complexity of the response to the stimulus of virus pneumonia are further shown by certain patients developing heterophile antibodies against sheep or fowl erythrocytes (Aaron 1952 Eyquem Cateigne Hannoun and Fanconnier 1953) and by some sera giving positive Wassermann and Kahn reactions (Florman and Weiss 1945 Kreis 1947 Aaron 1952)

Prognosis

The prognosis in hæmolytic anæmia following virus pneumonia is generally good for hæmolysis is essentially short lived Although some deaths have been recorded (Horstmann and Tatlock 1943 Finland *et al* 1945) most patients seem to recover completely Sacks Workman and Jahn (1952) stated that 33 out of 35 patients recovered completely

Pathogenesis

The pathogenesis of hæmolytic anæmia associated with auto antibody formation is dealt with in Chapter 11 There is however one point that should be made at this stage it was not clear at first whether or not sulphonamide drugs played a part in bringing about the hæmolytic episodes which followed virus pneumonia In most of the recorded cases one or other variety of these drugs had in fact been given before the onset of hæmolysis However acute hæmolytic anæmia has undoubtedly developed in patients to whom no sulphonamide drugs had been given at any time (e g Ginsberg 1946) It is thus reasonable to suppose that although drugs of the sulphonamide type can cause hæmolytic episodes (see Chapter 15) it is unlikely that they often play an important role in the causation of the hæmolytic anæmia which follows virus pneumonia

A high titre cold antibody was present in the patient s serum which rapidly hæmolysed normal erythrocytes at pH 6 5 to 7 0 up to a temperature of at least 30 C

The patient eventually made a complete clinical and hæmatological recovery

Case Report Acute Hæmolytic Anæmia following Virus Pneumonia Spontaneous Recovery

Case 16 The patient (M T) was a man aged 41 He was admitted into hospital with a history that three weeks previously he had developed an influenza like illness He had suffered from malaise weakness and fever and later developed an unproductive cough Three days before admission he suddenly became much weaker and seriously breathless When admitted into hospital on June 4th 195_ he was found to be extremely ill and dyspnœic on the slightest exertion He was markedly pale and slightly jaundiced

Physical Examination His cardiovascular system was normal except for some flame shaped hæmorrhages in the retinæ and slight œdema of the left leg Crepitations and rhonchi were to be heard in his right lung A radiograph of his chest revealed a small area of collapse at the right base The liver was just palpable but the spleen could not be felt The urine contained a trace of albumin and an excess of urobilin There were no other abnormal physical signs His temperature was 101 F

Laboratory Investigations On admission there were 2 200 000 erythrocytes per c mm with 7 1 g hæmoglobin per 100 ml and 7 5% reticulocytes the M C V was 96 c μ There were 1_ 000 leucocytes per c mm with 60% neutrophils and 660 000 platelets per c mm Stained films showed moderate anisocytosis slight poikilocytosis and some polychromasia and spherocytosis There were 6 normoblasts per 100 leucocytes The erythrocyte osmotic fragility was definitely increased lysis commenced in 0 60% saline the M C F being 0 455% NaCl The serum bilirubin concentration was 3 0 mg per 100 ml and the total serum proteins 6 9 g per 100 ml with 3 2 g albumin and 3 7 g globulin per 100 ml

Serology The direct antiglobulin test was positive the reaction being of the cold antibody type (see p 236) His blood rapidly underwent spontaneous auto agglutination after withdrawal this was reversed on warming at 37 C His serum contained a cold agglutinin with a relatively high thermal amplitude normal group O corpuscles were agglutinated to titres of 4 000 at 2 C 128 at 23 C and 4 at 35 C There was however no agglutination at 37 C Normal corpuscles were hæmolysed in the patient s serum acidified to between pH 6 5 and 7 0 at 20 C and at 30 C but not at 37 C Trypsinized normal corpuscles were hæmolysed but not agglutinated at 37 C to a titre of 4

P N H erythrocytes were hæmolysed by the patient s serum to a titre of 16 at 37 C and 256 at 20 C The indirect antiglobulin reaction was strongly positive when sensitization was carried out at 37 C and at 20 C the reaction however appeared to be no stronger at 20 C than at 37 C and the intensity of sensitization was only slightly increased by acidification Sensitization was abolished completely if the serum had been previously heated at 56 C for 30 minutes

200 000 units 3 hourly was started This too had no striking effect his temperature however reached normal by July 21st

On July 19th that is 14 days after the onset of his illness the patient was noticed to have become very pale and slightly jaundiced He complained of having been sweating profusely His liver was just palpable and tender but the spleen could not be felt The urine contained a trace of bile The hæmoglobin concentration was found to have fallen to 8 1 g per 100 ml and the patient s blood was observed to undergo rapid auto agglutination after withdrawal

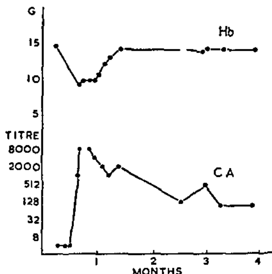


FIG 72 The rise and fall in the cold agglutinin titre and the accompanying changes in hæmoglobin concentration in a patient who developed an acute hæmolytic anæmia following virus pneumonia (Case 15) C A = cold agglutinin titre at 2 C

Serology The direct antiglobulin reaction was positive The cold agglutinin titre at 2 C was found to be 8 000 this titre was maintained for a few days then slowly subsided reaching a level of 128 three months later (Fig 72) His serum when acidified to *p*H 6 5 to 7 0 rapidly hæmolysed normal erythrocytes (Table 9) the thermal range of the antibody was so high that at first the antibody was thought to be of the warm type However it was later shown that although his serum hæmolysed normal corpuscles rapidly at room temperature and less readily at temperatures up to 30 C no lysis was caused at 37 C

The clinical and hæmatological recovery from this hæmolytic episode was rapid and by August 6th 1948 18 days after the onset of hæmolysis tests for hæmolysis *in vitro* were negative using normal corpuscles The cold agglutinin titre had by then fallen to 1 000

Summary A case of acute hæmolytic anæmia occurring during convalescence from a respiratory illness probably virus pneumonia

Summary A case of acute hæmolytic anæmia following a respiratory infection probably virus pneumonia Cold antibodies were present in unusually high concentration and were active *in vitro* up to 37 C Spontaneous recovery took place and the abnormal antibodies eventually disappeared

ACUTE HÆMOLYTIC ANÆMIA (AUTO ANTIBODY TYPE) ASSOCIATED WITH INFECTIOUS MONONUCLEOSIS

A small number of instances have been reported of an acute hæmolytic episode occurring during the course of an illness resembling infectious mononucleosis

Dameshek (1943) described a possible case and further probable examples have since been reported by Riva (1946) Petrides (1948) Ellis Wollenman and Stetson (1948) Wilson Ward and Gray (1949) Appelman and Morrison (1949) Small and Hadley (1950) Sawitsky Papps and Wiener (1950) Huntington (1951) Berté (1951) Mermann (1952) and Hall and Archer (1953)

Clinical Features The clinical features of this syndrome seem to be less uniform than in hæmolytic anæmia following virus pneumonia For one thing the hæmolytic episodes in most of the recorded examples have developed at about the same time as the signs of infectious mononucleosis and have not followed a clearly defined preceding illness as in the case of hæmolytic anæmia following virus pneumonia

The clinical symptoms and signs of infectious mononucleosis have been indefinite in most instances although sore throat and enlargement of lymph nodes and the spleen have usually been found at some stage in the illness Most of the patients were at first acutely ill with high fever and then became weak anæmic and jaundiced The patients of Ellis Wollenman and Stetson (1948) and Appelman and Morrison (1949) may have had hæmoglobinuria in most reports the urine has been said to contain urobilin but not bile

Laboratory Observations

Anæmia is usually moderately severe and spherocytes and increased fragility have been reported in most cases In the majority of patients the hæmolytic episode has been short lived and recovery has been associated with a marked reticulocytosis The maximum leucocyte counts varied according to the case reports cited above from 8 800 to 34 000 cells per c mm the percentage of lymphocytes ranging between 33% and 80% A

The antibody therefore appeared to be a cold one it was unusual in that its thermal range was remarkably high in relation to its only moderate activity at 2 C The effect of temperature on the different activities of the antibody was not uniform the agglutination of normal or trypsinized normal corpuscles was markedly potentiated by a fall in temperature but the degree of sensitization of normal corpuscles when tested for by means of antiglobulin serum seemed to be about the same whether the sensitizations were carried out at 37 C or at 20 C This

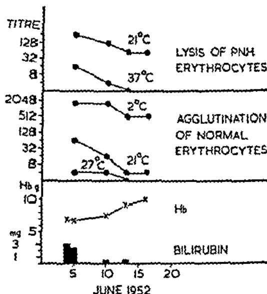


FIG 73 The changes in cold antibody concentrations in the serum of a patient recovering from an acute hæmolytic anæmia following virus pneumonia (Case 16)

discrepancy is unexplained A possible explanation is that two distinct fractions of antibody were involved His serum agglutinated *Strepto coccus* M G to a titre of 80

Subsequent Course The patient made a steady and uninterrupted recovery He was treated with Terramycin 500 mg 6 hourly for 9 days The sequence of changes in his blood state and the corre sponding serological changes are shown in Fig 73 Within 10 days of admission there was a slight diminution in the titre of cold agglutinins at 2 C and a substantial fall in titre at 21 C Similarly the ability of his serum to hæmolyse P N H erythrocytes at 37 C was rapidly lost It appeared therefore as if the loss of the antibody s ability to act at a relatively high temperature was the factor which was associated with clinical recovery

The patient was seen again 6 weeks after admission His cold agglu tinin titre was then 256 Three months after admission the titre was 64 clinically the patient had quite recovered

to a titre of 1 024 Cold agglutinins against human group O corpuscles were also present their titre at 4 C being 256 Agglutination also took place at room temperature and this did not completely disperse at 37 C Normal human erythrocytes suspended in fresh patient s serum and chilled at 4 C underwent lysis when subsequently incubated at 37 C (positive Donath Landsteiner test) This test was still positive 7 weeks later at a time when the patient had practically recovered it was negative when repeated 2 years later The Wassermann and Kahn tests were negative

The patient was critically ill for 2 weeks during which time he was transfused with 7 litres of blood Eventually he made a complete recovery

It is not possible from a study of the reports quoted above to obtain a clear picture of the nature of the antibodies responsible for the patients hæmolytic anæmia Cold agglutinins at high titres do not appear to be regularly formed and it may well be that the actual type of antibody differs from patient to patient

It has to be admitted that infectious mononucleosis was not *proved* to be the inciting agent of the hæmolytic episodes in any of the cases referred to above However in some of the cases at least it is difficult to suggest a satisfactory alternative cause or an alternative diagnosis to infectious mononucleosis Moreover the infective agent of infectious mononucleosis certainly has the power to stimulate antibody production Although this is characteristically heterophile other serum changes which result in positive Wassermann and Kahn reactions sometimes occur as they do following virus pneumonia Whether the development of auto antibodies is due to infection with a variant of the normal virus or due to an unusual antibody response on the part of the patient remains to be seen

HÆMOLYTIC ANÆMIA FOLLOWING OTHER VIRUS INFECTIONS

Influenza A Laroche Milliez Dreyfus Dausset and Leprat (1951) described a patient who developed an acute hæmolytic episode in the course of influenzal pneumonia Serological tests indicated influenza type A infection The onset of the patient s anæmia was related to the formation of high titre cold antibodies and it seems difficult to exclude the possibility of a concurrent infection with the virus of virus pneumonia

Newcastle Disease Moolten and Clark (1952a and b) and Moolten and co workers (1953) have claimed to have isolated the virus of Newcastle disease (N D V) from the blood stream of

large proportion of the lymphocytes were said to be typical of infectious mononucleosis

Serology Heterophile agglutinins at raised titres have been found in the sera of all the patients at some time in their illness often diminishing in titre with the patients recovery In the reports of Small and Hadley (1950) Berté (1951) Huntington (1951) and Hall and Archer (1953) it is specifically stated that the anti sheep cell agglutinins were *not* absorbed by guinea pig kidney i e the antibodies behaved as does the antibody of infectious mononucleosis

Investigations for the presence of anti human erythrocyte antibodies have been less regularly and thoroughly carried out Dameshek (1943) reported that an auto agglutinin was present in the serum of his patient which was most active at room and ice box temperature Appelman and Morrison (1949) on the other hand stated that tests for cold agglutinins and the Donath Landsteiner test were negative Sawitsky Papps and Wiener (1950) reported that both the direct and indirect antiglobulin tests were positive Berte (1951) stated that autohæmolysins were present active at room temperature but gave no details tests for cold agglutinins were negative Mermann (1952) on the other hand observed a rise in the cold agglutinin titre of his patient s serum from 128 to 2 048 Hall and Archer (1953) reported the presence of auto hæmagglutination and mentioned that the direct antiglobulin test was positive

The observations of Ellis Wollenman and Stetson (1948) appear to be unique The history of their patient suggested virus pneumonia but the blood picture was in favour of a diagnosis of infectious mononucleosis the antibody apparently resembled the Donath Landsteiner antibody (see p 276)

Their patient was a man aged 21 years who was admitted into hospital with a history of having passed bloody urine for the previous 3 days and of having become weak and breathless on exertion Two weeks previously he had had an upper respiratory tract infection associated with headache and anorexia for which he had no treatment

On examination he was found to be pale and slightly icteric The liver and spleen were just palpable and were tender An enlarged lymph node was palpated in the left auricular region but elsewhere his lymph nodes did not seem enlarged His temperature was 99 4 F

The day following admission an acute hæmolytic episode developed hæmoglobinuria was intense and the patient s hæmoglobin concentration fell from 12 2 g per 100 ml to 5 9 g per 100 ml in 5 hours The total leucocyte count rose to 24 300 cells per c mm 64% of the cells being lymphocytes corresponding in type to those seen in infectious mononucleosis Agglutinins against sheep erythrocytes were present

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certain patients suffering from acquired hæmolytic anæmia The possible role of this virus in the causation of hæmolytic anæmia is considered in Chapter 11 (p 295)

Coxsackie Virus A Betke Richarz Schubothe and Vivell (1953) reported isolating the Coxsackie A virus from the fæces of a boy aged three years who was suffering from an acute hæmolytic anæmia They were also able to show that the patient s serum contained anti viral neutralizing antibodies in high concentrations during his convalescence The possible role of this virus in the causation of acquired hæmolytic anæmia is also considered in Chapter 11

Measles Virus An example of acute hæmolytic anæmia associated with the formation of a cold antibody of the Donath Landsteiner type which appeared to follow an attack of measles is referred to in Chapter 10 (p 288)

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CHAPTER 9

ACQUIRED HÆMOLYTIC ANÆMIA (AUTO ANTIBODY TYPE)

III THE SPECIFICITY AND REACTIONS *IN VITRO* OF THE AUTO ANTIBODIES

IN this chapter further details will be given of the specificity of the antibodies of acquired hæmolytic anæmia and of their reactions *in vitro* The nature of the antibodies will also be briefly discussed The reactions of the warm and cold varieties of antibody will be dealt with separately

WARM ANTIBODIES

Species Specificity The limited data available suggest that warm antibodies are specific for the human species although they may react to some extent with the erythrocytes of chimpanzees and Rhesus monkeys

Sturgeon (1947) obtained an antibody from the erythrocytes of a patient suffering from acquired hæmolytic anæmia by heat-elution and found that the antibody reacted with human erythrocytes of group O (Rh positive and Rh negative) group A group B and group AB but not with Rhesus monkey cells or sheep cells

Kidd (1949) tested eluates made from erythrocyte stromata obtained from four patients with acquired hæmolytic anæmia Human erythrocytes of groups A B O Rh positive and O Rh negative became strongly sensitized to an anti human globulin serum when treated with the eluates but mouse guinea pig rabbit rat fowl sheep and horse corpuscles were not affected Rhesus monkey erythrocytes were weakly sensitized by two of the eluates Similar observations were made by Komninos and Rosenthal (1953) in a larger series of cases they failed however to demonstrate agglutination or sensitization of monkey corpuscles

Wiener Gordon and Gallop (1953) tested the serum of one patient against the erythrocytes of various species using cells which had been acted upon by the enzyme ficin Chimpanzee and Rhesus monkey erythrocytes were agglutinated to slightly lower titres than were human corpuscles spider monkey cow horse and sheep erythrocytes were agglutinated to far lower titres Wiener and co workers pointed out that in this respect the behaviour of the antibody of their patient paralleled that of Rh antibodies

RIVA C (1946) Zur Frage der Autoagglutination der roten Blut korperchen erworbener hamolytischer Ikterus bei Drusenfieber *Helv med Acta* 13 446

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SACKS M S WORKMAN J B and JAHN E F (1952) Diagnosis and treatment of acquired hemolytic anemia *J Amer med Ass* 150 1556

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globulin tests were in fact positive in both Dameshek s and Davidsohn and Oyamada s cases

The author believes that Dameshek s interpretation is more likely to be the correct one at least in most instances To prove Davidsohn and Oyamada s contention that strictly auto specific antibodies existed it would seem to be necessary to elute the antibodies from the patients corpuscles and then to test the eluates for the specificity of the antibodies they contained This was not carried out Davidsohn and Oyamada s interpretation seems open to argument for another reason if the antibody really was auto-specific in any particular case then normal corpuscles should survive for the normal length of time after transfusion to the patient This if it occurs at all must be a very rare event in acquired hæmolytic anæmia Indeed one of Davidsohn and Oyamada s own patients (Case 10) whose antibody was considered to be strictly auto specific had been given 100 blood transfusions in five months

As has just been mentioned evidence has been recently forth coming which indicates that *specific* auto antibodies may in fact be formed by patients suffering from acquired hæmolytic anæmia However the antibodies have *not* been strictly auto specific the ones so far identified have been mostly directed against Rh anti gens particularly the antigen e The first example of this type of specificity was probably observed by Dr Ruth Sanger in an unpublished fatal case of acquired hæmolytic anæmia In 1953 Weiner and co workers described a further patient in whose serum anti e was demonstrated The corpuscles of this patient gave a positive direct antiglobulin reaction and eluates made from the corpuscles were shown to contain an antibody reacting only with e positive corpuscles As the patient s probable genotype was CDe/CDe *there seemed no doubt that the anti e was acting as an* auto antibody and was presumably responsible for the patient s hæmolytic anæmia Hollander (1953) has also reported the finding of a specific auto antibody in a patient of probable Rh genotype CDe/cde suffering from acquired hæmolytic anæmia The patient s serum contained anti c whilst anti c and a non specific component were identified in an eluate made from his corpuscles

Dacie and Cutbush (1954) have published the results of a detailed investigation into the specificity of the warm antibodies developed by ten patients suffering from idiopathic acquired hæmolytic anæmia Several interesting facts emerged One patient out of the ten failed to develop non specific antibodies Instead she formed both anti e and anti C as her probable genotype was known to be CDe/cde these antibodies also could be justifiably described as specific auto antibodies The patient died of a fulminating hæmolytic anæmia her history is *described* on p 202 (Case 12) All the other nine patients formed non

Specificity in Relation to Human Erythrocytes An essential feature of the antibodies of acquired hæmolytic anæmia is their ability to act upon the patient s own corpuscles i e they act as auto antibodies About this there is no dispute The antibodies in most instances at least also act as iso antibodies and react with most if not all human erythrocytes to some extent Until recently the antibodies have been generally considered to be non specific that is to say they were thought to react with an antigen of undetermined nature present on the surface of all human corpuscles which was quite independent of the presence or absence of other blood group antigens It is now known that this is not necessarily true and that auto antibodies of definite specificity usually within the Rh system may be formed by some patients in addition to unidentifiable ' non specific components (see below) That there might be differences in the sensitivity of different samples of normal corpuscles to the antibodies has been known for some time but the significance of this in relation to a possible specificity of the antibodies had not been fully appreciated

Denys and van den Broucke (1947) described how blood from 32 subjects was tested for compatibility with their patient s serum by means of the indirect antiglobulin test Twenty six of the samples gave weak reactions whilst two samples were not sensitized The reactions with a more active serum derived from a second patient were similar However the exact specificity of the antibodies in relationship to known blood group factors could not be determined moreover it appeared that all the samples of blood which reacted feebly or failed to react were from females

Kuhns and Wagley (1949) found an unidentifiable antibody in their patient s serum which agglutinated the patient s erythrocytes as well as 63% of a panel of normal erythrocytes apparently irrespective of their blood groups as far as they were then known

More recently it has been suggested that antibodies reacting only with the patient s own corpuscles may sometimes be formed Davidsohn and Oyamada (1953) reported that when the sera of patients suffering from acquired hæmolytic anæmia were tested for warm agglutinins using 20% bovine albumin as a diluent three consistent reaction patterns were observed (1) in which antibodies in the patients sera agglutinated normal corpuscles to approximately the same titre as the patients own corpuscles (2) in which the patients corpuscles were agglutinated to significantly higher titres than were the normal corpuscles and (3) in which the patients corpuscles were agglutinated but normal corpuscles not agglutinated (auto-specific antibodies) Dameshek (1951) had previously made similar observations but concluded tentatively that the stronger reactions with the patients corpuscles were due to the cells being already coated with antibody and that the presence of serum and albumin led to their agglutination The direct anti

equally complex and tends to vary from patient to patient The differences between the reactions of the antibodies of different patients are no doubt reflections of the individual character of each patient s antibody response

In the following sections further details will be given of the antiglobulin reaction in acquired hæmolytic anæmia and of the agglutination and hæmolysis of enzyme treated corpuscles by patients sera

Antiglobulin Reaction

Dacie (1951) showed that there was a striking difference between the antiglobulin reactions of corpuscles sensitized by anti D and cold antibodies respectively It was found that whereas the agglutination of cells sensitized by anti D was readily inhibited if very small amounts of human γ globulin were added to the anti globulin serum (γ-globulin type of reaction) very much more γ globulin was required to inhibit the agglutination of cells sensi tized by cold antibodies (cold antibody type of reaction) It was concluded that whereas Rh antibodies were probably γ globulins and reacted with an anti-γ-globulin in the antiglobulin serum as previously suggested by Coombs and Mourant (1947) the cold antibodies might not be γ globulins (see later) Similar observa tions were made by Renton (1952) It was also shown (Dacie 1951 1953) that the reaction with the auto antibodies of the majority of patients with acquired hæmolytic anæmia of the warm antibody type was similar to if not identical with that of cells sensi tized by anti D both in respect of the effect of γ globulin and in the occurrence of an inhibitory zone when a highly potent anti globulin serum was used (see van Loghem *et al* 1950 Hubinont and Massart Guiot 1950 Hubinont 1951) Twenty four patients have been investigated up to the time of writing the reactions were of the γ-globulin type in sixteen in four of the remaining patients the reactions seemed identical with those of cold antibodies (see p 236) and in the other four patients the reactions were intermediate between the γ globulin and cold antibody types The reaction of one patient of the last group (Case 8) was notable for its inconstancy Typical reactions are illustrated in Tables 10 and 11

When it was possible to compare direct reactions with the indirect reactions obtained by sensitizing normal corpuscles in the patients sera both reactions generally behaved identically in respect of the *effects of γ globulin and in the intensity of the reaction in different* concentrations of antiglobulin serum There was one exception the

specific antibodies in addition however three of them formed anti e, and one patient anti e and anti D (at different times) As the probable Rh genotype of the latter four patients was in each instance CDe/cde it was clear that these antibodies too were capable of acting as auto antibodies Indeed in two cases anti e was successfully recovered from the eluates made from the patients corpuscles

The non specific antibodies were equally interesting In three cases using erythrocytes of genotype -D-/-D- (Race Sanger and Selwyn 1951) it was possible to show that the non specific antibody consisted of two components (1) non specific antibody in the strict sense i e an antibody reacting with and adsorbed by erythrocytes of all groups and types tested including the -D-/ D- corpuscles and (2) an unidentified component reacting with and adsorbed by all corpuscles tested except -D-/-D- ones the -D-/-D- corpuscles presumably being deficient in some antigen common to other types of corpuscles in addition to being deficient in Cc and Ee

More recently van Loghem and van der Hart (1954) have published the results of studies carried out on ten patients with acquired hæmolytic anæmia the antibodies being of the warm type in six of them Specific auto antibodies were identified in five cases the specificities being anti D anti c anti c + e (two cases) and anti Jk[a] respectively

In view of the undoubted presence of specific Rh antibodies in some cases (as referred to above) it is interesting to note that Wiener and Gordon (1953) and Wiener Gordon and Gallop (1953) have suggested that the auto antibodies in typical acquired hæmolytic anæmia might be directed against the nucleus of the Rh Hr substance This hypothesis was based on finding that erythrocytes maximally sensitized with anti D and the auto antibodies of acquired hæmolytic anæmia reacted to the same titre with antiglobulin sera and also on the observed reactions with erythrocytes of various species already mentioned on p 231 Dacie (1951 1953) also pointed out that erythrocytes sensitized by anti D and the auto antibodies of acquired hæmolytic anæmia respectively often reacted identically in the antiglobulin test

In vitro Reactions of the Warm Antibodies of Acquired Hæmolytic Anæmia

In the previous section enough has been said to indicate how complex and variable is the specificity of the warm type of antibody the behaviour of the antibodies *in vitro* appears to be

indirect reaction given by the patient s serum was of the γ-globulin type but the direct reaction was of the intermediate type Whether an *intermediate reaction means the adsorption of more than one type of* antibody protein has not been determined nor has the specificity or nature been established of the warm antibodies which appear to react with antiglobulin serum in a manner similar to that of cold antibodies

It is probably significant that all the auto-antibodies shown to have a relationship or be identical with Rh antibodies behaved in the anti globulin reaction like anti D (Dacie and Cutbush 1954) However too much weight should not be given to a strict correlation between the specificity of an antibody and the reactions in antiglobulin serum of cells sensitized by the antibody The author tested four samples of the specific iso-antibody anti Fy^a although three of the antibodies reacted as if they were γ globulins the reaction of the fourth (that of Mr Duffy himself) was of the cold antibody type

Effect of Inactivation by Heat Heating a patient s serum at 56 C for 30 minutes has the same effect on the warm antibodies of acquired hæmolytic anæmia as it has on sera containing anti D sensitization is not abolished although it may be slightly diminished in intensity *This is in strong contrast to the complete inhibition of sensitization* which results from the action of heat on sera containing cold antibodies (see p 259)

Agglutination of Trypsinized Erythrocytes

The warm antibodies of acquired hæmolytic anæmia seem to be almost invariably capable of agglutinating at 37 C normal erythrocytes treated with enzymes such as trypsin Agglutination takes place readily in saline dilutions of the patients sera in some cases however as in the agglutination of trypsinized corpuscles by anti D (Hoyt and Zwicker 1952) the agglutination titre may be increased two or four fold if the titrations are carried out in normal serum rather than in saline Acidification of the serum to pH 6 5 to 7 0 may raise the titre still higher (Table 12) Inhibition of agglutination (zoning) in serum containing high concentrations of antibody may occur but is rarely seen Agglutination is irreversible and attains its maximum intensity after about two hours incubation (cf p 245)

Methods using trypsinized corpuscles should be looked upon as being complementary to the antiglobulin method and not a substitute for it for in some patients antibodies may be demonstrable using trypsinized cells which are not detectable by the use of anti globulin serum As already referred to the serum of one of the author s patients (To) who had recovered from an acute hæmolytic episode contained an apparently non specific antibody which agglutinated trypsinized corpuscles of all groups to a high titre (256) but failed to sensitize normal corpuscles to antiglobulin

TABLE 10 *The effect of the addition of human γ globulin to a rabbit anti human globulin serum on the ability of the latter to agglutinate the erythrocytes of patients suffering from acquired hæmolytic anæmia*

Cases 9 10 K_1 *and* R_1 *were suffering from acquired hæmolytic anæmia of the warm antibody type and Cases* 13 *and* 14 *from acquired hæmolytic anæmia of the cold antibody type Case* 18 *was suffering from paroxysmal cold hæmoglobinuria The results with corpuscles sensitized by incomplete anti D and incomplete anti H respectively are shown for comparison*

Case number or antibody	Type of antibody (W = warm) (C = cold)	Dilutions of 4% γ globulin solution					
		1 in 4	1 in 16	1 in 64	1 in 256	1 in 1 024	Control (saline)
Anti D	W	0	0	0	0	++	+++
9	W	0	0	0	0	+++	+++
10	W	0	0	0	0	trace	++
K_1	W	trace	±	±	+	+±	++±
R_1	W	trace	+	+±	++	+++	+++
Anti H (normal incomplete cold antibody)	C	±	++	+++	+++	+++	+++
13	C	±	+	++	++	++	++
14	C	+	++	++	++	++	++
18	C	++	++	++	++	++	++

+++ denotes strong agglutination ± weak but definite agglutination
+ +± ++ and ++± denote intermediate grades of agglutination

TABLE 12 *The agglutination of normal erythrocytes and of trypsinized normal erythrocytes by the serum of Case 9 and the effect of different diluents and pH*

Erythrocytes	Diluent	pH	Dilutions of patient's serum (Case 9)				
			1 1	1 in 4	1 in 8	1 in 16	Control
Normal	Saline	8 0	0	0	0	0	0
	Normal serum	8 0	0	0	0	0	0
	Acid normal serum	6 5	0	0	0	0	0
Trypsinized normal	Saline	8 0	++	trace	0	0	0
	Normal serum	8 0	++	++	trace	0	0
	Acid normal serum	6 5	++	++	+	trace	0

++ denotes strong agglutination + weaker but definite agglutination

TABLE 11 *The effect of diluting an antiglobulin serum on its ability to agglutinate the erythrocytes of patients suffering from acquired hæmolytic anæmia*

Cases 9 10 K_1 and R_1 were suffering from acquired hæmolytic anæmia of the warm antibody type and Cases 13 and 14 from acquired hæmolytic anæmia of the cold antibody type Case 18 was suffering from paroxysmal cold hæmoglobinuria The results with corpuscles sensitized by incomplete anti D and incomplete anti H respectively are shown for comparison

Case number or antibody	Type of antibody (W = warm) (C = cold)	Dilutions of antiglobulin serum					
		1 in 4	1 in 16	1 in 64	1 in 256	1 in 1024	Control (saline)
Anti D	W	+	+±	**++**	+	±	0
9	W	++	+++	**++++**	+++	+±	0
10	W	±	+	**++**	**++**	±	0
K_1	W	+±	**++**	+±	+	±	0
R_1	W	++	**++±**	++	±	0	0
Anti H (normal incomplete cold antibody)	C	**++**	+	trace	0	0	0
13	C	**++**	+	trace	0	0	0
14	C	**+++**	+±	trace	0	0	0
18	C	**++**	+±	±	trace	0	0

+++ denotes strong agglutination ± weak but definite agglutination + +± ++ and ++± denote intermediate grades of agglutination

The optimum dilution of the serum is marked □ (shown in bold above)

TABLE 12 *The agglutination of normal erythrocytes and of trypsinized normal erythrocytes by the serum of Case 9 and the effect of different diluents and pH*

Erythrocytes	Diluent	pH	Dilutions of patient serum (Case 9)				
			1 in [illegible]	1 in 4	1 in 8	1 in 16	Control
Normal	Saline	8·0	0	0	0	0	0
	Normal serum	8·0	0	0	0	0	0
	Acid normal serum	6·5	0	0	0	0	0
Trypsinized normal	Saline	8·0	+ +	trace	0	0	0
	Normal serum	8·0	+ +	+ +	trace	0	0
	Acid normal serum	6·5	+ +	+ +	+	trace	0

+ + denotes strong agglutination + weaker but definite agglutination

TABLE 11 *The effect of diluting an antiglobulin serum on its ability to agglutinate the erythrocytes of patients suffering from acquired hæmolytic anæmia*

Cases 9 10 K1 *and* R1 *were suffering from acquired hæmolytic anæmia of the warm antibody type and Cases* 13 *and* 14 *from acquired hæmolytic anæmia of the cold antibody type Case* 18 *was suffering from paroxysmal cold hæmoglobinuria The results with corpuscles sensitized by incomplete anti D and incomplete anti H respectively are shown for comparison*

C se number or antibody	Type of antibody (W = warm) (C = cold)	D lutions of antiglobulin serum					
		1 in 4	1 in 16	1 in 64	1 in 56	1 in 1 0 4	Contro (alin)
Anti D	W	+	+±	**++**	+	±	0
9	W	++	+++	**++++**	+++	+±	0
10	W	±	+	**++**	**++**	±	0
K1	W	+±	**++**	+±	+	±	0
R1	W	++	**++±**	++	±	0	0
Anti H (normal incomplete cold antibody)	C	**++**	+	trace	0	0	0
13	C	**++**	+	trace	0	0	0
14	C	**+++**	+±	trace	0	0	0
18	C	**++**	+±	±	trace	0	0

++++ denotes strong agglutination ± weak but definite agglutination + +± ++ and ++± denote intermediate grades of agglutination

The optimum dilution of the serum is marked [box]

TABLE 13 *The reactions* in vitro *of the serum of a patient (To) with normal erythrocytes of the probable genotypes* CDe/CDe cDE/cDE *and* cde/cde *The serum contained anti* L *and anti* c *as well as a non specific antibody acting on trypsini ed corpuscles The figures in brackets refer to agglutinin titres*

Prob bl g type of ythrocytes	Aggluti ti by antigl bull erum	Aggl ti ti i rum alb min	Aggl tinati f trypsi ized n rmal rythrocytes	
			(*B fore* bsorption with CDe/CDe co puscles)	(*After* bsorpti with CDe CDe corpus les)
CDe/CDe	0	0	+++ (250)	0
cDE/cDE	+++	++ (16)	+++ (230)	++ (32)
cde/cde	++	+ (4)	++ (64)	+ (8)

serum The reactions of this serum which also contained two immune iso antibodies anti c and anti E are illustrated in Table 13

The case mentioned above is clearly exceptional but Dacie and Cutbush (1954) in investigating ten patients found that the antibodies of four of them (non specific antibodies and anti e) reacted preferentially with trypsinized corpuscles and that anti e and anti D obtained from eluates of a further patient s corpuscles could only be demonstrated using trypsinized corpuscles

Dausset (1952b) has made some rather similar observations He referred to four patients suffering from acquired hæmolytic anæmia and cirrhosis of the liver in whom reactions using trypsinized cells were positive although the direct and indirect antiglobulin tests were negative The patients own erythrocytes when trypsinized underwent marked agglutination in autogenous plasma to which a one fourth volume of 20% albumin had been added Normal corpuscles when trypsinized were also agglutinated by the patients plasma particularly in the presence of bovine albumin Dausset believed that his patients had formed doubly incomplete antibodies which he suggested might have two non reactive valencies

Foster and Hutt (1953) have recently made some interesting observations on a possibly non specific antibody in the serum of a patient suffering from Hodgkin s disease and hæmolytic anæmia Normal group O corpuscles were agglutinated at 37 C in both saline and albumin dilutions of the patient s serum to approximately the same titres However when trypsinized corpuscles were used it was found that although strong agglutination followed the use of crystalline trypsin no agglutination took place when comparable amounts of crude trypsin or chymotrypsin were used instead of the crystalline variety This was thought to be due to the antigen sites being destroyed by the chymotrypsin The exact specificity of the antibody was unfortunately not determined

Less commonly the warm antibodies of acquired hæmolytic anæmia although sensitizing normal corpuscles to antiglobulin serum fail to agglutinate trypsinized corpuscles This was observed in three out of the 30 patients investigated by the author using normal corpuscles acted upon by crystalline trypsin as described by Dacie and de Gruchy (1951)

The reactions of one of these patients may be quoted as an example Normal corpuscles sensitized in her serum or in an eluate made from her corpuscles were agglutinated quite strongly by an antiglobulin serum but the same corpuscles when trypsinized were only very weakly agglutinated by the patient s serum and not agglutinated at all by the eluate made from the patient s corpuscles The antibody in this case appeared to be a non specific one

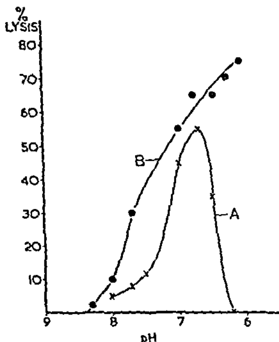


FIG 74 The effect of pH on the hæmolysis of normal erythrocytes by a warm hæmolysin in the serum of a patient suffering from acquired hæmolytic anæmia (Dacie 1949a)
A = effect on observed hæmolysis
B = effect on the adsorption of the antibody

trypsinized corpuscles but do not sensitize cells to the antiglobulin test see p 237) It seems therefore probable that this type of hæmolysin is additional to and separate from the actual antibodies which are responsible for erythrocyte destruction in vivo

It was especially interesting to find this type of warm hæmolysin in four patients suffering from hæmolytic anæmia of the cold antibody type—in one patient the hæmolytic anæmia was secondary to virus pneumonia in the other three patients it was of the idiopathic type

The serum of one of these latter patients (Case 13) has been studied the most the lytic factor being present in approximately the same concentration in many samples of serum examined over a three year period The factor appears to be non specific The optimum pH for lysis is between pH 6 4 and 7 0 but the range of pH within which

Warm Hæmolysins

As already mentioned (p 185) warm hæmolysins have but rarely been reported in the sera of patients suffering from acquired hæmolytic anæmia It should be added that the mere demonstration of rapid autohæmolysis *in vitro* is no proof that the hæmolysis is brought about by complement and antibody Rapid autohæmolysis may be due for instance to the rapid lysis of spherocytes A distinguishing feature is that the lysis of spherocytes will take place even if complement is inactivated as for instance when anticoagulants such as heparin are added to the blood (e g Cases 11 and 12) Nor can the presence of a warm hæmolysin as opposed to the cold variety of more frequent *occurrence be considered to be proved unless the tests in vitro* have been carried out with sera and erythrocyte suspensions which have been carefully warmed to 37 C before mixing a point of technique which has seldom been mentioned

However it can hardly be doubted that warm hæmolysins do exist The author has studied one patient whose serum contained an antibody capable of hæmolysing normal corpuscles at an acid *p*H (Dacie 1949a) in addition to several other patients whose sera contained hæmolytic factors demonstrable by means of trypsinized corpuscles and/or P N H erythrocytes but not by normal corpuscles (Table 7 p 192)

The hæmolysin in the serum of the patient reported by Dacie (1949a) was thermostable although barely active in unacidified serum it caused definite and rapid lysis of the patient s corpuscles or of normal corpuscles in the presence of complement if the serum was suitably acidified The effect of *p*H is shown in Fig 74 the optimum *p*H for hæmolysis was about *p*H 6 7 with inhibition above *p*H 8 0 and below *p*H 6 2 Actually the inhibition at *p*H 6 2 and below appeared to be due to inhibition of complement as the lysin itself seemed to be adsorbed increasingly well as the serum was made more acid (Fig 74) This patient s serum even if unacidified hæmolysed trypsinized normal erythrocytes to a titre of 256 and P.N H erythrocytes to a titre of 64 at 37 C

The sera of six other patients caused minor or major degrees of hæmolysis of trypsinized normal corpuscles at 37 C three (unacidified) sera also caused the hæmolysis of P N H corpuscles but none of them hæmolysed normal corpuscles even if acidified to *p*H 6 5 to 7 0

The significance nature and specificity of the factors which hæmolyse trypsinized or P N H erythrocytes but which do not *cause the lysis of normal unmodified corpuscles* are unknown They are presumably of little pathogenetic importance (they appear to be similar in this respect to antibodies which agglutinate

two hæmolytic factors were present in different proportions it seems at least as likely that a subtle difference in the "fit" of the hæmolysin in relation to the receptor surfaces of the different kinds of erythrocytes is the explanation

The hæmolytic factors described in the preceding paragraphs probably differ significantly from at least two other factors capable of hæmolysing trypsinized normal corpuscles or P N H erythrocytes at 37° C One type of lysin is the cold antibody the thermal range of which just reaches 37 C (see p 223) the other type was referred to by Dacie and de Gruchy (1951) as occurring in the serum of a patient (not suffering from hæmolytic anæmia) who was in clinical and hæmatological remission after receiving treatment for pernicious anæmia with vitamin B_1 This lytic factor was recently described in more detail by Hurley and Dacie (1953) At 37 C it hæmolysed trypsinized normal corpuscles but not P N H corpuscles it was thermolabile being apparently destroyed by heating at 56 C for 5 minutes and it was active only between a narrow *p*H range (*p*H 6 6 to 8 5)

This hæmolytic factor appears to be similar in properties to the reversible agglutinin which Rosenthal and Schwartz (1951) demonstrated to be present in many normal sera but with the difference that the serum of the patient studied by Hurley and Dacie contained a far greater concentration of the factor than usual It can be distinguished from the hæmolysins found in the sera of patients with acquired hæmolytic anæmia by its thermolability its more restricted *p*H range its specificity for trypsinized erythrocytes (it should be added however that several sera from patients with acquired hæmolytic anæmia lysed trypsinized but not P N H erythrocytes) by the agglutination it causes being reversible and by its not being associated with hæmolytic anæmia

Antilytic Effect of Normal Serum Dameshek and Schwartz (1938) and Farrar Burnett and Steigman (1940) reported that normal serum was capable of neutralizing the hæmolysins present in the sera of the patients they studied The significance of these observations is uncertain The author has not observed any major degree of inhibition by normal serum of the lysis of trypsinized corpuscles by warm hæmolysins the sera have in fact been titrated using fresh normal human serum as diluent that of Case 13 being noteworthy in not causing any lysis unless diluted with normal serum owing to its very low content of complement In one patient (Case 21 p 340) however substantially less lysis but not inhibition resulted when the patient's serum was diluted with an equal volume of normal serum instead of with saline

Inhibition of Agglutination by Normal Serum There are indications that normal human serum may inhibit other manifestations of antibody action in certain cases Denys and van den Broucke (1947) reported that the sensitization of normal corpuscles to antiglobulin serum by the serum of the patient they investigated was slightly diminished

it is active is comparatively wide (Fig 75) The lytic factor is thermostable and withstands heating for 30 minutes at 56 C and lysis of *both* trypsinized and P N H erythrocytes can be shown to be accompanied by utilization of complement

Other patients in whom warm hæmolysins active against trypsinized corpuscles have been identified have been described by Dausset (1952a) and Rosenthal Komninos and Dameshek (1953) The patient referred to by Dausset (1952a) was an elderly man suffering from carcinoma of the prostate and hæmolytic anæmia in his serum were identified an incomplete warm antibody a cold agglutinin at a titre of 512 and a

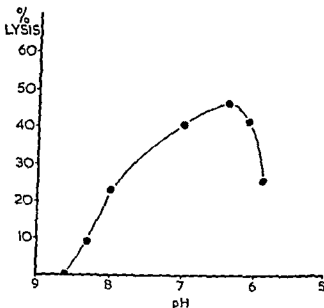


FIG 75 The effect of *p*H on the hæmolysis of trypsinized *normal* erythrocytes by a warm hæmolysin in the serum of a patient suffering from an idiopathic acquired hæmolytic anæmia (Case 13)

hæmolytic factor active against trypsinized corpuscles The patient of Rosenthal and co workers (1953) was a woman aged 33 years Several *different* antibody components were found in her *serum* including a hæmolysin active against trypsinized corpuscles but not *against* normal corpuscles and lipoid antigen antibodies giving a positive Kahn test

Subtle differences between the hæmolytic factors in the sera of *different* patients were described by Dacie and de Gruchy (1951) They showed for instance that when two sera were titrated side by side one serum lysed P.N H erythrocytes to a higher titre *than trypsinized normal* corpuscles whilst with the other serum exactly the opposite happened It is not thought that this necessarily means that

against human sera known to contain cold agglutinins active against human erythrocytes at pathologically raised titres (1-8 to 2 048). In most cases the observed titres against the animal corpuscles seemed to be independent of the presence or absence of cold antibodies active against human cells. However agglutination of monkey erythrocytes occurred more regularly and in higher titres in sera containing anti human cold antibodies in raised concentrations; reversal at 37 C was usually incomplete. Absorption experiments showed that whereas absorption with rabbit corpuscles resulted in a significant fall in the titres against human and guinea pig corpuscles as well as against autologous cells absorption with human or guinea pig corpuscles had little effect on the cold agglutinin titres for heterologous cells.

These observations were extended by Millet and Fincler (1946) who found that it was impossible to absorb cold agglutinins from human serum by means of rabbit guinea pig or ox cells. They showed however that this specificity depended upon surface antigens for if erythrocyte stromata were used instead of intact corpuscles species specificity disappeared.

Wiener Gordon and Gallop (1953) studied three patients whose sera contained cold antibodies in raised concentrations. All three patients had an acquired hæmolytic anæmia in two of them this was secondary to lymphoblastoma or lymphomatosis. The antibodies were found to react strongly with Rhesus monkey spider monkey pig and rabbit erythrocytes but only weakly or not at all with chimpanzee cow horse or sheep cells.

Specificity of Reactions with Human Erythrocytes There is probably still much to be learnt about the reactions with different types of human corpuscles. It is already clear though that it is an over simplification to refer to the antibodies as non specific without any qualification. For one thing there is an as yet incompletely defined relationship with the ABO group system in respect of the agglutinability of corpuscles by some varieties of cold antibodies. Moreover there are certain well known antibodies of definite specificity such as α_1 and α_2 and anti P which act as cold antibodies and which may be confused with cold antibodies of less definite specificity (Dockeray and Sachs 1941).

There are indications in the literature that group O erythrocytes are often more strongly agglutinated than corpuscles of groups AB A or B. This was so with the serum investigated by Boxwell and Bigger (1931) and with seven out of nine sera investigated by Finland and co workers (1945). On the other hand Turner and Jackson (1943) and Young (1946) found no significant differences.

Stratton s (1943) observations were more remarkable. He investigated five sera four of them from patients with infections or malignant disease and one from a patient suffering from acquired hæmolytic anæmia with Raynaud s phenomena. The

by the addition of normal serum Whether this is a constant phenomenon has yet to be established More remarkable were the observations made by Heni and Blessing (1952) on a patient suffering from chronic lymphatic leukæmia complicated by hæmolytic anæmia This patient s serum contained an apparently non specific agglutinating factor active in saline dilutions to a titre of more than 32 000 at 0 C and 256 at 42 C However it was inhibited particularly at higher temperatures by the presence of fresh normal human serum with the result that no auto agglutination took place at 37 C although the erythrocytes underwent spontaneous agglutination in saline The factor in normal serum inhibiting agglutination was identified as an albumin The nature of the most unusual agglutinating factor is unknown its activity was largely dependent upon the presence of thermolabile fractions of complement The patient s serum itself was deficient in both γ globulin and complement

COLD ANTIBODIES

Species Specificity Several studies have been carried out on the reactions of the erythrocytes of different species to the cold antibodies present in the serum of patients recovering from virus pneumonia or suffering from acquired hæmolytic anæmia of the cold antibody type No clear picture emerges It appears that although the cold antibodies are mainly specific for the human species there may be a certain amount of cross reaction especially with monkey rabbit and guinea pig erythrocytes

Clough and Richter (1918) studied a patient who almost certainly had suffered from virus pneumonia and found that the patient s serum contained cold agglutinins against rabbit guinea pig hen sheep cat and pig cells as well as against human corpuscles absorption with either rabbit or human erythrocytes removed the agglutinins acting on both types of cell

Turner and Jackson (1943) carried out a more extensive study on the sera of seven patients Heterospecific cold agglutinins active against rabbit mouse guinea pig horse and sheep corpuscles were present in each serum but equally powerful hetero antibodies were found in normal sera not containing high titre cold agglutinins active against human erythrocytes Eluates into warm saline obtained from human corpuscles sensitized in the patients sera in the cold were next tested for specificity rabbit and human cells were found to be agglutinated to about the same titre and guinea pig and pig erythrocytes to low titres On the other hand absorption experiments showed that the anti human agglutinins could be removed without affecting to any significant degree the agglutination of the heterologous erythrocytes except possibly that of guinea pig corpuscles

Further studies were carried out by Finland Peterson and Barnes (1945) Like Turner and Jackson they found that heterospecific agglutinins existed in many normal human sera and that of the species they studied rabbit corpuscles were agglutinated to the highest titres Finland Peterson and Barnes also tested a panel of animal corpuscles

than were the group O and B corpuscles the group A_1 corpuscles behaved in an intermediate way The differences in sensitivity were however slight and not at all comparable with the differences reported by Crawford Cutbush and Mollison (1953) in respect of the incomplete cold antibodies found in normal sera H positive and H negative human saliva respectively were added to the eluates but no convincing evidence was obtained that the antibodies were inhibited by H substance at the concentrations normally present in saliva

Cold Agglutinins

Titre and Thermal Range Cold agglutinins may exist in patients sera in relatively enormous concentrations being detectable in rare instances as for instance in Case 14 in sera diluted 1 in 64 000 or even more using normal corpuscles and carrying out the titrations at 2 C It is characteristic of cold antibodies that the agglutinin titre is sharply reduced with rise

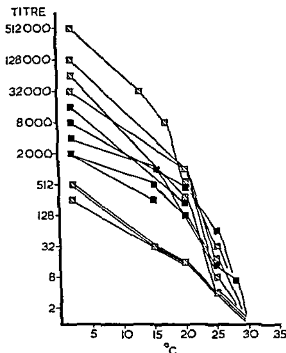


FIG 76 Relationship between temperature and cold agglutinin titre

Cases of acquired hæmolytic anæmia of the cold antibody type

■ Case of hæmolytic anæmia following virus pneumonia

agglutinating antibodies in three of the sera behaved exactly as if they had anti O (H) specificity the antibodies in the other two sera seemed to be non specific Amongst the latter was the serum of the patient with acquired hæmolytic anæmia which agglutinated A_1 cells and O cells to identical titres

Bird (1951a) investigated a patient with acquired hæmolytic anæmia whose serum contained an apparently non specific cold agglutinin He found that if this serum was absorbed with the patient's own (group AB) corpuscles it then ceased to agglutinate O A and B cells as well as autologous cells However when the serum was absorbed with group O cells so that it was no longer active against O cells agglutinins persisted which still reacted with A B and the patient's cells Bird's experiments in fact suggested that this particular serum was a mixture of anti O anti A anti B and anti patient's cell components A similar combination of components was stated to be present in seven other group AB sera studied in the same way

Later Bird (1951b) reported that when four group AB sera containing cold agglutinins were absorbed with group O cells the subsequent titres against A and B cells actually increased as did their thermal amplitude Whether this means that anti O agglutinins may in some way block activity against A and B cells remains to be seen If this were so it would provide a possible explanation for the lower titres reported in some cases against A and B cells (e g Stratton 1943) More recent work (Bird 1953) indicates however that absorption with group O corpuscles usually removes all the supposed components of a cold panagglutinin Bird suggested that cold auto agglutinins possess multiple combining sites which may render it impossible to separate the various components (if in fact they exist)

Crawford Cutbush and Mollison (1953) have also shown that differences between corpuscles in sensitivity to apparently non specific cold antibodies may depend on ABO group differences Working with the normally occurring incomplete cold antibody (Dacie 1950b) they were able to show that the antibody had anti H specificity the sensitivity of normal corpuscles to the antibody being in proportion to their H content i e O cells were strongly sensitized A_2 cells slightly less strongly B cells moderately strongly and A_1 cells weakly A_1B cells were not sensitized

The author has studied the cold antibodies in the sera of ten patients with acquired hæmolytic anæmia of the cold antibody type with particular reference to their reactions with corpuscles of different ABO groups However no definite evidence of specificity was obtained with any of the sera

Group O corpuscles were sensitized in the patients' sera in the cold and then washed several times in ice cold saline Saline eluates were then made at 37° C The ability of the antibodies contained in these eluates to cause agglutination and sensitization to antiglobulin serum was tested using corpuscles of groups O A_2 A_1 and B respectively On the whole group A_1 corpuscles appeared to be slightly less sensitive

than were the group O and B corpuscles the group A_2 corpuscles behaved in an intermediate way The differences in sensitivity were however slight and not at all comparable with the differences reported by Crawford Cutbush and Mollison (1953) in respect of the incomplete cold antibodies found in normal sera H positive and H negative human saliva respectively were added to the eluates but no convincing evidence was obtained that the antibodies were inhibited by H substance at the concentrations normally present in saliva

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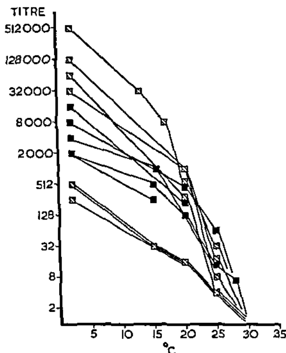


FIG 76 Relationship between temperature and cold agglutinin titre
Cases of acquired hæmolytic anæmia of the cold antibody type
Cases of hæmolytic anæmia following virus pneumonia

in temperature usually the greatest reduction in the activity of antibodies present in pathological concentrations takes place as the temperature is raised from 20 to 25 C (Fig 76) Almost always agglutination (of normal corpuscles) is abolished at a temperature of 30 to 32 C Exceptionally antibodies are encountered with a relatively low titre at 2 C but at the same time with a high thermal range In the author s experience antibodies of this type are rare in acquired hæmolytic anæmia (see Case 21)

Effect of pH Variation in *p*H between the range 8 5–6 0 makes little difference to the agglutinin titre when a serum containing cold antibodies is titrated using normal corpuscles In most sera however the intensity of agglutination in strong concentrations of serum is reduced by acidification to *p*H 6 5

Agglutination of Trypsinized Normal (T N) Erythrocytes and P N H Corpuscles As already mentioned T N erythrocytes are very susceptible to agglutination by cold antibodies The apparent agglutinin titre of a serum is raised fourfold or more by their use and the thermal range of the antibody extended upwards P N H corpuscles are not agglutinated more strongly than are normal corpuscles but as will be demonstrated subsequently they very readily undergo hæmolysis in sera containing cold antibodies

Cold Hæmolysins

The Effect of pH Using Normal Erythrocytes It is usually possible to demonstrate that a serum containing cold agglutinins at a titre of 1 000 or over at 2 C is capable of causing the lysis of normal corpuscles Nevertheless even with very high titre sera little or no hæmolysis of normal corpuscles may be demonstrable *in vitro* if the sample of serum is collected and manipulated without regard for loss of carbon dioxide Under these circumstances the *p*H of the serum will rise to about *p*H 8 0 However if the *p*H is readjusted to the physiological level (*p*H 7 3–7 4) by the addition of acid some hæmolysis can often be demonstrated The optimum reaction for hæmolysis is usually in the region of *p*H 6 5 to 6 8 hæmolysis is inhibited at the acid side of *p*H 6 0 (Dacie 1950a) (Fig 77 (A))

The adsorption of a typical acid hæmolysin increases steadily with diminishing *p*H at any rate as far as *p*H 5 8 (Fig 77 (B)) In unacidified serum no adsorption appears to take place However this is actually more apparent than real for hæmolytic antibody can be demonstrated in eluates made from corpuscles sensitized in the cold in unacidified serum (see p 255)

The experiment illustrated in Fig 77 shows that the amount of hæmolysin absorbed from serum apparently increases with increasing

acidity Nevertheless if a pH hæmolysis curve is constructed (Fig 77 (A)) it is usually observed that little or no hæmolysis takes place below pH 6 This is apparently due to inhibition of the hæmolytic action of serum complement below pH 6 as is demonstrated in Fig 77 (C)

The effects of pH on hæmolysis as described above and as illustrated in Fig 77 are not entirely constant Although acidification generally increases hæmolysis and is usually essential for the hæmolysis of normal corpuscles exceptions in which acidification has less effect or even results in some inhibition of hæmolysis have been encountered

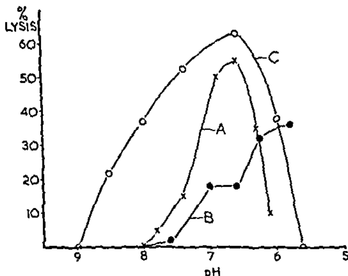


FIG 77 The effect of pH on the hæmolysis of normal erythrocytes by a high titre cold antibody

A = effect on observed hæmolysis
B = effect on the adsorption of the hæmolysin
C = effect on the action of human serum complement

For instance whilst the hæmolytic antibodies in the sera of all seven patients suffering from hæmolytic anæmia after virus pneumonia were of the typical acid type the antibodies in two out of the six sera obtained from the patients suffering from idiopathic acquired hæmolytic anæmia with Raynaud's phenomena were atypical In one patient (Case 1 of Fernman *et al* 1951) although the amount of hæmolysis of normal corpuscles was increased by acidification a moderate amount was regularly caused by unacidified serum The other serum was more unusual still for it hæmolysed normal corpuscles at 20 C more actively at pH 8 0 than at pH 6 5 (Table 14) A similar case to this was published by Bonnin (1954)

in temperature usually the greatest reduction in the activity of antibodies present in pathological concentrations takes place as the temperature is raised from 20 to 25 C (Fig 76) Almost always agglutination (of normal corpuscles) is abolished at a temperature of 30 to 32 C Exceptionally antibodies are encountered with a relatively low titre at 2 C but at the same time with a high thermal range In the author s experience anti bodies of this type are rare in acquired hæmolytic anæmia (see Case 21)

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The adsorption of a typical acid hæmolysin increases steadily with diminishing *p*H at any rate as far as *p*H 5 8 (Fig 77 (B)) In unacidified serum no adsorption appears to take place However this is actually more apparent than real for hæmolytic antibody can be demonstrated in eluates made from corpuscles sensitized in the cold in unacidified serum (see p 255)

The experiment illustrated in Fig 77 shows that the amount of hæmolysin absorbed from serum apparently increases with increasing

TABLE 15 *The relative sensitivity of normal and P N H erythrocytes to hæmolysis by a high titre cold antibody (the serum of Case 3 of Ferriman* et al 1951) *and the effect of* pH

Erythr ytes	pH	Dil ti ns of patl t s serum					
		1/10	1/100	1/1 000	1/10 000	1/100 000	Co t ol
Normal	8 0 6 5	0 ±	0 0	0 0	0 0	0 0	0 0
P N H	8 0 6 ↄ	++ ++	++++ ++++	+++ +++	++ +++	± ++	0 0

++++ denotes almost complete lysis +++ ++ and ± lesser degrees of lysis

TABLE 14 *The effect of pH on the hæmolysis of normal erythrocytes by the sera of two patients Sm and Mo suffering from acquired hæmolytic anæmia of the cold antibody type*

Case	pH	Dilutions of patient's serum						
		1 in 2	1 in 4	1 in 16	1 in 64	1 in 256	1 in 1 024	Control
Sm	8 0 6 5	0 0	0 ++	0 ++	0 +	0 trace	0 0	0 0
Mo	8 0 6 5	0 0	0 0	++ ++	+± +	+ trace	± 0	0 0

++ denotes marked hæmolysis + and ± less marked but definite hæmolysis

TABLE 15 *The relative sensitivity of normal and P N H erythrocytes to hæmolysis by a high titre cold anti-body (the serum of Case 3 of Ferriman et al, 1951) and the effect of pH*

Erythrocytes	pH	Dilutions of patient's serum					
		1/10	1/100	1/1 000	1/10 000	1/100 000	Control
Normal	8.0 6.5	0 ±	0 0	0 0	0 0	0 0	0 0
P N H	8.0 6.5	++ ++	++++ ++++	+++ +++	++ +++	± ++	0 0

++++ denotes almost complete lysis, +++, ++ and ± lesser degrees of lysis

TABLE 14 *The effect of pH on the hæmolysis of normal erythrocytes by the sera of two patients Sm and Mo, suffering from acquired hæmolytic anæmia of the cold antibody type*

Case	pH	Dilutions of patient's serum						
		1 in	1 in 4	1 in 16	1 in 64	1 in 256	1 in 1 0 4	Control
Sm	8 0 6 5	0 0	0 ++	0 ++	0 +	0 trace	0 0	0 0
Mo	8 0 6 5	0 0	0 0	++ ++	+± +	+ trace	± 0	0 0

++ denotes marked hæmolysis + and ± less marked but definite hæmolysis

Table 16 *The effect of heating sera containing high titre cold antibodies on the ability of the sera to cause the hæmolysis of normal and P.N H erythrocytes respectively*

I Hæmolysis after 1 hour at 17 C

II After washing in saline the corpuscles left unhæmolysed at the end of I and then re suspending in fresh normal serum and incubating at 37 C for 1 hour

The sera were acidified (pH 6 5) *when tested with the normal corpuscles but unacidified* (pH 8 0) *when tested with the P N H corpuscles*

Cas	S rum	Erythrocytes			
		N mal		P.N H	
		I	II	I	II
1 a	Heated Not heated	0 ++	++	0 ++	++
Ma	Heated Not heated	0 0	trace ++	0 +	+
Sl	Heated Not heated	0 0	0 trace	0 +	+
Da	Heated Not heated	0 ++	0	0 ++	+
14	Heated Not heated	0 ++	+	0 ++	++

++ denotes marked hæmolysis and + a definite but lesser degree of hæmolysis
denote no observation

P N H corpuscles succeed in doing so even from unacidified inactivated sera However it can be shown by making eluates into warm saline from normal corpuscles exposed to patients inactivated sera that potentially hæmolytic antibodies are in fact adsorbed in the cold irrespective of whether the serum is fresh or inactivated or unacidified or acidified to *p*H 6 5 (Table 17) It seems that heating affects the apparent adsorption of the anti bodies by destroying the thermolabile components of complement and that it is the destruction of complement that leads to a partial or complete failure of firm union between the normal erythrocyte surface and antibody with the result that antibody is rapidly eluted from the cells when the temperature is raised

Using Trypsinized Normal (T N) Corpuscles and P N H Erythrocytes As with normal corpuscles acidification typically increases both the amount of hæmolysis observed and the apparent titre of the serum However whereas hæmolysis is generally not discernible at all when normal corpuscles are suspended in un acidified patients sera hæmolysis readily takes place in unacidified sera when T N or P N H corpuscles are substituted The effect of *p*H is illustrated in Table 15 The very greatly increased sensitivity of P N H corpuscles to hæmolysis by this type of antibody as compared with normal erythrocytes is also well shown

The Effect of Heating and the Need for Complement The cold antibodies causing hæmolysis are thermostable i e they are not destroyed by heating at temperatures of 50 C to 58 C for as long as 30 minutes However as has been observed with hæmo lysins of the Donath Landsteiner type (see p 276) the presence of fresh unheated serum is more or less essential for the firm adsorption of the hæmolytic antibody when corpuscles are sensi tized in the cold The serum components necessary for antibody adsorption and hæmolysis appear to be components of serum complement (see later)

It is easy to demonstrate that complement is removed from serum when normal corpuscles are hæmolysed by cold antibodies at the appropriate *p*H and temperature In one experiment the complement titre was significantly reduced when the hæmolysis test was carried out at room temperature (17 C) and the serum acidified to *p*H 6 5 There was also probably minor fixation of complement in unacidified serum at 17 C but no fixation in acidified or unacidified serum at 37 C or 2 C

The relative importance of the presence of complement during sensitization at room temperature (17 C) was investigated using five different sera The results are recorded in Table 16 In every instance except one (Case Pa) more hæmolysis was produced when the test cells were chilled in the presence of fresh serum than when the serum had been previously inactivated by heating at 56 C Moreover in at least two instances no hæmolysis resulted when normal corpuscles after being sensitized at 17° C in inactivated serum were subsequently incubated at 37 C in fresh normal serum Nevertheless the differences between sera after inactivation appeared to be quantitative rather than qualita tive for when trypsinized normal (T N) corpuscles or more especially P N H erythrocytes were used the inimical effect on lysis of being *sensitized in heated serum was always overcome to a greater* or lesser extent

The results recorded in Table 16 suggest at first sight that normal corpuscles may fail to adsorb hæmolytic antibodies from acidified inactivated sera (e g in Cases Sl and Da) whereas

TABLE 16 *The effect of heating sera containing high titre cold antibodies on the ability of the sera to cause the hæmolysis of normal and P.N H erythrocytes respectively*

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II After washing in saline the corpuscles left unhæmolysed at the end of I and then re suspending in fresh normal serum and incubating at 37 C for 1 hour

The sera were acidified (pH 6 5) *when tested with the normal corpuscles but unacidified* (pH 8 0) *when tested with the P N H corpuscles*

Case	Serum	Erythrocytes			
		Normal		P N H	
		I	II	I	II
Pa	Heated	0	++	0	++
	Not heated	++		++	
Ma	Heated	0	trace	0	+
	Not heated	0	++	+	
Sl	Heated	0	0	0	+
	Not heated	0	trace	+	
Da	Heated	0	0	0	+
	Not heated	++		++	
14	Heated	0	+	0	++
	Not heated	++		++	

++ denotes marked hæmolysis and + a definite but lesser degree of hæmolysis
denotes no observation

P N H corpuscles succeed in doing so even from unacidified inactivated sera However it can be shown by making eluates into warm saline from normal corpuscles exposed to patients inactivated sera that potentially hæmolytic antibodies are in fact adsorbed in the cold irrespective of whether the serum is fresh or inactivated or unacidified or acidified to pH 6 5 (Table 17) It seems that heating affects the apparent adsorption of the anti bodies by destroying the thermolabile components of complement and that it is the destruction of complement that leads to a partial or complete failure of firm union between the normal erythrocyte surface and antibody with the result that antibody is rapidly eluted from the cells when the temperature is raised

TABLE 17 *The antibody content of a saline eluate prepared from erythrocytes sensitized at 2 C in fresh and in heat inactivated samples of a serum containing high titre cold antibodies (Case 3 of Ferriman* et al 1951)

Source of eluate	Agglutinati n (A) or hæmoly is (H)	Dilution of El tes 1 in 8	1 in 3	1 in 1 8	1 in 512	Control
Corpuscles sensitized in patient s *fresh* serum	A	+	±	trace	0	0
	H	+	+	±	0	0
Corpuscles sensitized in patient s *heated* serum	A	+	±	±	0	0
	H	+	+	±	0	0

\+ denotes agglutination or hæmolysis
± denotes weak but definite agglutination or hæmolysis

Normal erythrocytes were used for the agglutinin titration and P N H erythrocytes for the hæmolysin titration

The degree of failure of firm fixation of antibody seems to vary from one serum to another and as has already been mentioned P N H erythrocytes and to a lesser extent trypsinized normal corpuscles can adsorb antibody effectively even from heated sera

Using complement fractions [1] of human serum and P N H corpuscles it could be shown that C'1 and C'2 and C'4 (and presumably C'3) were all required for the fixation of the antibody and hæmolysis C'1 was found to be more important than C'2 in the cold phase and C'2 and C'4 to be essential for hæmolysis in the warm phase C'4 did not seem to be essential in the cold phase nor C'1 in the warm phase The role of C'3 was not determined P N H erythrocytes were also found to adsorb a certain amount of antibody at 2 C in the complete absence of complement fractions (the necessity or otherwise of C'3 was not determined) for hæmolysis took place on warming provided that the cells were then suspended in whole unacidified fresh normal serum

Human serum has generally been employed as a source of complement in experiments of the type summarized above Guinea pig serum will serve but it is less satisfactory than human serum when P.N.H. corpuscles are used because it contains heterolysins to which these cells are extremely sensitive

The Effect of Temperature on the Adsorption of Hæmolytic Cold Antibodies Using Normal Corpuscles The upper thermal limit at which a hæmolytic effect can be demonstrated corresponds quite closely with the highest temperature at which agglutination takes place As already mentioned it has been possible in all the cases studied to demonstrate hæmolysis at 20 C and in most cases even at 30 C

It has been repeatedly observed however that little or no hæmolysis may follow chilling suspensions at 0 to 2 C before they are warmed at 37 C whereas duplicate suspensions not cooled below 15 C may undergo marked hæmolysis It seems possible that this is due to the more intense agglutination at 2 C preventing the adsorption of complement and the consequent firm fixation of the antibody on to the cells

The simplest and most practical way of demonstrating the hæmolytic activity of the antibodies is undoubtedly to set up suspensions in acidified (and unacidified) serum at room temperature (15 to 20 C) This temperature is suitable for the adsorption of the antibodies and not cold enough to inhibit the lytic activity of complement Schubothe (1953) treating the antibody as a monophasic lysin showed that his patient s serum caused maxi

[1] Complement fractions of human serum were generously supplied by Dr R R A Coombs C'1 and C'2 were prepared by ammonium sulphate precipitation followed by dialysis and C'4 by ammonia inactivation as described by Coombs Blomfield and Fulton Roberts (1950)

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Source of eluate	Aggl ti tion (A) or hæmoly is (H)	Dilution of Eluates				
		1 in 8	1 in 3	1 in 1 8	1 in 51	Control
Corpuscles sensitized in patient s *fresh* serum	A	+	±	trace	0	0
	H	+	+	±	0	0
Corpuscles sensitized in patient s *heated* serum	A	+	±	±	0	0
	H	+	+	±	0	0

+ denotes agglutination or hæmolysis
± denotes weak but definite agglutination or hæmolysis

Normal erythrocytes were used for the agglutinin titration and P N H erythrocytes for the hæmolysin titration

mum lysis at 23 C at this temperature lysis commenced within 10 seconds of adding the cell suspension to the serum

Incomplete Cold Antibodies

Dacie (1950b) reported that an incomplete type of antibody could be demonstrated to be present by means of the antiglobulin test in many if not in all normal sera As already mentioned these antibodies have anti H specificity (Crawford Cutbush and Mollison 1953) Dacie referred to the fact that the antibodies could only be detected in fresh serum and that the presence of anticoagulants as well as heat inactivation prevented sensitization He also reported that the incomplete antibodies once adsorbed in the cold remained adherent to the erythrocytes even if they were repeatedly washed in saline at 37 C despite the fact that cold agglutinins were readily eluted by this procedure Antibodies with generally similar but not identical properties are present at high concentrations in sera containing high titre cold agglutinins

The Effect of Temperature on Elution of Incomplete Cold Antibodies It can be shown that incomplete antibodies of both the normal and pathological types are not readily eluted unless the temperature of the saline in which the corpuscles are suspended is raised above 48 C Moreover repeated washing at 37 C removes no more antibody than does washing in saline at 15 C

The actual agglutination by antiglobulin sera of corpuscles which have adsorbed incomplete cold antibodies takes place at least as well at 37 C as at lower temperatures

The Effect of Anticoagulants The effect of anticoagulants in inhibiting the adsorption of incomplete cold antibodies is illustrated in Table 18

The Effect of Heating at 56 C If a normal serum is heated at 56 C for as little as 5 minutes it loses its power of coating corpuscles with incomplete cold antibodies This is also true of pathological sera in which cold antibodies are present in greatly raised concentrations

The effect of heating is not satisfactorily explained on the hypothesis that incomplete antibodies are thermolabile It is possible to restore at least partially the activity of a heated serum by the addition to it of a fresh serum from which the naturally occurring incomplete cold antibodies have been absorbed (Table 20) However *complete* restoration of activity has never been observed as the result of adding a fresh serum to a heated serum It is therefore possible that heating at 56 C does in fact result in partial destruction of the antibody or alternatively that heating causes some inhibition of adsorption in a more subtle way

TABLE 18 *The effect of anticoagulants on the ability of a normal serum containing an incomplete cold anti-body to sensitize normal erythrocytes to agglutination by an antiglobulin serum*

Anticoagulants	Concentration of anticoagulant (mg per 100 ml)	Agglutination by antiglobulin serum
Heparin	1.0	+
	0.5	++
	0.25	+++
	0.125	++++
Ammonium and potassium oxalate mixture	2.0	0
	0.5	+++
	0.125	++++
Trisodium citrate	6.0	++
	1.5	+++
Control (no anticoagulant)	0	++++

++++ denotes very strong agglutination +++ ++ and + lesser degrees of agglutination

The apparent titre of an incomplete cold antibody is raised significantly by titrating the serum in a fresh normal serum containing little or no incomplete cold antibody instead of in saline This is illustrated in Table 19 The results indicate that the presence of some component or components of fresh serum is necessary for the effective adsorption of incomplete cold antibodies The failure of the serum of Case Ro to bring about sensitization when titrated in saline is particularly interesting and appears to be correlated with its very low complement content (10 units normal range 70–150 units)

Erythrocytes strongly sensitized by incomplete cold antibodies as judged by the antiglobulin reaction do not undergo agglutination when suspended in albumin containing media Nor does the adsorption of incomplete antibodies prevent agglutination or hæmolysis if the cells are subsequently exposed to high titre cold antibodies

The Role of Complement Experiments with complement fractions have confirmed that the factor in normal serum required for the effective adsorption of incomplete cold antibodies is identical with serum complement Both the thermolabile fractions C 1 and C 2 and the stable fraction C 4 (and C 3) are required (Table 20) The role of complement is similar to but not identical with its function in the adsorption of cold hæmolysins for whereas with incomplete antibodies the presence of complement seems essential with cold hæmolysins the need is not absolute and may be overcome (see p 255)

It again seems possible that complement acts by securing firm irreversible fixation of the incomplete antibody on to the erythrocyte surfaces thereby preventing its elution when the cells are warmed subsequently It is interesting to find that with high titre cold antibodies incomplete antibody may be readily recovered in warm saline eluates of sensitized cells suggesting that more antibody is adsorbed than can be firmly bound It can in fact be recovered as readily in warm saline eluates of corpuscles sensitized in heat inactivated serum as it can from eluates prepared from corpuscles sensitized in fresh serum This indicates that complement plays no part in the adsorption of reversibly bound antibody

The Effect of pH H ion concentration has a marked effect on the adsorption of incomplete cold antibodies With normal serum maximal adsorption seems to take place in unacidified serum (at about pH 8 0) there is inhibition at a pH greater than 9 and at a pH less than 6 (Table 21) However with sera containing cold antibodies at pathological concentrations the maximum adsorption

TABLE 19 *Titration with normal erythrocytes of incomplete cold antibodies diluted in saline and in normal serum respectively*

Serum	Diluent	Serum dilutions					
		1 in 4	1 in 16	1 in 64	1 in 256	1 in 1024	Control
Normal	Saline	++	trace	0	0	0	0
	Serum	++	+	0	0	0	0
Virus pneumonia	Saline	+++	++	+	0	0	0
	Serum	++++	+++	++	+	+	trace
Idiopathic acquired hemolytic anemia (Case Ro) (cold antibody type)	Saline	0	0	0	0	0	0
	Serum	++++	++++	+++	++	++	trace

++++ denotes very strong agglutination by an antiglobulin serum
+++ ++ and + denote lesser degrees of agglutination

TABLE 21 *The effect of pH on the sensitization to an antiglobulin serum of normal erythrocytes by a normal cold antibody and a pathological high titre cold antibody*

Serum	pH							
	9.3	8.9	8.5	8.0	7.7	7.1	6.5	5.7
Normal (anti H)	0	+	+++	+++	+++	++	+	0
High titre cold antibody	0	0	±	+	+++	++++	++++	+

++++ denotes very strong agglutination
+++ ++ + and ± lesser degrees of agglutination

TABLE 20 *The effect of the presence of the various fractions of complement on the sensitization of normal erythrocytes by a serum containing high titre cold antibodies (Case 3 of Ferriman* et al 1951)

a	Fractions of compl ment	Aggl tin tion by ntiglobul n serum
Patient s fresh serum	C 1 C 2 C 3 C 4	++++
Patient s heat inactivated serum	C 3 C 4	0
Patient s heat inactivated serum + Normal fresh serum	C 1 C 2 C 3 C 4	+++
Patient s heat inactivated serum + C 1 and C 2 fractions	C 1 C 2 C 3 C 4	+
Patient s ammonia treated serum	C 1 C 2 C 3	0
Patient s ammonia treated serum + Normal fresh serum	C 1 C 2 C 3 C 4	+++

++++ denotes very strong agglutination
+++ and + lesser degrees of agglutination

warm antibody in contrast to the naturally occurring iso anti bodies such as anti A and anti B which although acting at 37 C are considerably more active at lower temperatures In animals too the same distinction seems to hold in rabbits repeatedly inoculated with sheep or horse erythrocytes the thermal optimum for the agglutinins they produce rises as immunity develops and eventually passes 37 C (Millet and Hubinont 1946) Neverthe less in man at least cold antibodies develop in the course of what appears to be an immune response to infection (e g after virus pneumonia) Even so it is the rise in their thermal range toward body temperature which is the cause of their pathogenicity

The mechanism of the effect of temperature is obscure it is possible to conceive that the antibodies consist either of a mixture of molecules with different capacities for being adsorbed according to temperature or of a single type of molecule with groupings the activity of which is modified by temperature Filliti Wurmser and Jacquot Armand (1947) believed the latter hypothesis to be correct Wiener (1951) suggested that cold antibodies were normally heterogenetic in origin and that in consequence the antibodies were less perfectly adapted to the human antigens he concluded that such imperfect antigen antibody complexes might well dissociate as the temperature was raised

Warm auto antibodies do not seem to be formed in health cold auto antibodies undoubtedly are (Kettel 1929 Stats and Wasserman 1943 Dacie 1950b) The origin of these naturally occurring antibodies is uncertain and it is an attractive hypothesis to suppose that the antibodies present in disease at abnormally high concentrations are similar to those present in health at much lower concentrations However as has been already discussed a distinction between the two types of incomplete antibody can be made if the effect of pH is studied and also probably on the basis of their specificity Moreover as Crawford and Mollison (1951) have shown the two types of antibody react with distinctly different components of anti human globulin sera

Chemistry of Auto antibodies in Acquired Hæmolytic Anæmia

Antibodies in general are now regarded as specially modified serum globulins with molecular weights of the same order as that of the greater part of the normal serum globulins In human serum they usually have electrophoretic mobilities correspond ing with that of the γ globulins or of proteins lying between the β globulin and γ globulin (Marrack and Hoch 1949)

As already referred to it seems likely that the warm antibodies

in most cases seems to take place at approximately pH 6 5 to 7 0, the optimum being similar to that for hæmolysis by cold antibodies (Fig 77) The increased sensitization resulting from acidification is not entirely consistent but is generally more marked than in the case of warm antibodies (Table 21)

Prozones in Strong Concentrations of Antiglobulin Sera As mentioned previously van Loghem Stallman and Hart (1951) reported that inhibition of agglutination was not observed when strong concentrations of potent antiglobulin sera were used to agglutinate corpuscles which had been sensitized by the incomplete cold antibody present in the serum of the patient they studied The author s own observations lead him to believe that some inhibition of agglutination may be observed if *weak* suspensions of corpuscles coated with incomplete cold antibodies are suspended in high concentrations of a potent antiglobulin serum It is clear however that inhibition is much less obvious than with the warm antibodies of acquired hæmolytic anæmia it is usually not seen at all if 10 to 20% suspensions of cells are employed for the test (Table 11 p 238)

The effect produced by the addition of γ globulin to the antiglobulin serum on the reaction between an antiglobulin serum and corpuscles sensitized by cold antibodies has also been referred to previously The reaction of erythrocytes sensitized with cold antibodies is relatively insensitive to inhibition by γ globulin (Table 10 p 236) Presumably the antibody on the cell surfaces does not react with an anti-γ globulin component in the rabbit serum as the warm type of antibody nearly always seems to do

The Nature of the Auto antibodies of Acquired Hæmolytic Anæmia

The term antibody has been used many times in the preceding pages This has been for convenience and because there seems to be no simple alternative rather than because the author believes that the globulins causing autosensitization of the erythrocytes of patients suffering from acquired hæmolytic anæmia are necessarily specific antibodies in the sense that they have been developed as immune responses to antigens on the patients erythrocytes This may be so but it has not been proved in most instances In some cases at least it is possible that the proteins which damage erythrocytes are but part of an outpouring of globulin produced by an abnormal antibody forming tissue (see under *Ætiology* Chapter 11)

The Effect of Temperature The distinction between cold and warm antibodies has been made many times in the preceding pages It has been generally considered that warm antibodies are immune antibodies For instance anti Rh (Levine Katzin and Burnham 1940) and immune anti A are specific types of

puscles to hæmolysis can be overcome to some extent if the serum is suitably acidified and by the observation that abnormal corpuscles such as paroxysmal nocturnal hæmoglobinuria (P N H) erythrocytes and trypsinized corpuscles hæmolyse much more easily and quickly It is probably especially significant that with cold antibodies the hæmolysin titre using P N H erythrocytes often approximates closely to the agglutinin titre, the same is true of P N H erythrocytes and anti A iso antibodies which also cause agglutination of normal corpuscles readily but hæmolysis much less easily (Dacie 1949b)

Incomplete Antibodies The separability of incomplete cold antibodies from cold agglutinins and hæmolysins rests on the demonstration that in the presence of complement a certain amount of antibody is not eluted when sensitized corpuscles are repeatedly washed in saline at 37 C A smooth suspension of cold antibody sensitized cells can be readily obtained Such cells are agglutinated by antiglobulin sera Whether or not the antibody left adsorbed on the cells exists in the serum as a separate entity or whether it represents a moiety of the cold antibody molecule stuck on the surface of the erythrocytes through the agency of complement remains to be determined

The abnormal warm antibodies found in patients with acquired hæmolytic anæmia exist almost always in incomplete forms An agglutinin acting in saline at 37 C is rarely observed and hæmolytic activity is difficult to demonstrate Trypsinized corpuscles however are readily agglutinated and in a minority of cases a hæmolytic component can be revealed by the use of P N H or trypsinized erythrocytes There is certainly no exact parallelism between the antibody titres as estimated by the agglutination of trypsinized erythrocytes and the hæmolysin titres as estimated with P N H or trypsinized corpuscles Most of the antibodies in fact fail to cause hæmolysis under what appear to be favourable conditions others such as that in the serum of Case 13 constantly cause more hæmolysis of trypsinized corpuscles than agglutination It seems probable therefore that the antibody which commonly agglutinates trypsinized cells is distinct from the component which causes hæmolysis Evidence has already been produced (p 241) for the existence in some sera of antibodies capable of causing agglutination of trypsinized corpuscles but not of sensitizing normal corpuscles to antiglobin serum

The whole question of the behaviour *in vitro* and of the specificity and nature of the abnormal antibodies formed in patients suffering from acquired hæmolytic anæmia is without doubt an

of acquired hæmolytic anæmia are usually but not invariably γ globulins as judged by their reaction with antiglobulin serum neutralized or partially neutralized with human γ globulin and as shown by electrophoretic studies carried out on eluted antibodies (Young and Miller 1953) Incomplete cold antibodies on the other hand behave in the antiglobulin reaction as if they were not γ globulins or at any rate not normal γ globulins Further evidence on this point is required Stats Perlman Bullowa and Goodkind (1943) concluded from an electrophoretic study that the high titre *cold agglutinin in the serum* of their patient was in fact *a γ globulin* and a similar conclusion was reached by Spaet and Kinsell (1953) Gordon (1953) who carried out a more elaborate immuno chemical study considered however that the cold agglutinin he studied was not identical with normal serum γ globulin

The Unitary Nature of Non specific Antibodies

Another unsettled problem is concerned with the different effects that antibodies can be shown to exert on erythrocytes *in vitro* Early concepts of the nature of antibodies postulated that agglutinins hæmolysins and opsonins etc were separate substances (*see Browning* 1931 Zinsser Enders and Fothergill 1939) Later the view that the different manifestations of antibody reaction might be explained by a single immune substance acting under different environmental conditions gained ground (Dean 1917) A good deal of evidence is now available in support of the latter concept (Marrack 1938 Zinsser Enders and Fothergill 1939) The behaviour *in vitro* of the antibodies of acquired hæmolytic anæmia is partly in accord with this conception

It seems most likely that agglutinin hæmolysin' is one antibody and that incomplete antibody is a distinct and frequently encountered variant If cold antibodies are titrated using normal corpuscles the agglutinin and hæmolysin titres will be found to be strikingly different For example normal corpuscles may be agglutinated at 2 C by an antibody diluted 1 in 2 000 yet undergo only a trace of hæmolysis in the antibody diluted 1 in 4 under apparently optimal conditions This difference is apparently due to the natural insensitivity of human erythrocytes to complement hæmolysin lysis compared with their sensitivity to agglutination it is less easily explained on the hypothesis that an *agglutinin is present in high concentration* and a hæmolysin in low concentration It is possible that it is the surface structure of the normal human erythrocyte which is the barrier to hæmolysis This is supported by the fact that the insensitivity of normal cor

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extraordinarily complicated one Many patients seem to form more than one component of antibody at the same time and it is hardly an exaggeration to say that the antibody reactions of no two patients are exactly the same if a variety of methods of investigation are employed

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tion to the ætiological relationship between syphilis and paroxysmal hæmoglobinuria

The disease was the subject of a monograph by Chvostek in 1894 and by the end of the nineteenth century its clinical features were fairly well known although nothing was known of its pathogenesis

In 1904 Donath and Landsteiner published their classical paper They studied three patients and showed that the hæmolysis was in all probability due to an autohæmolysin which united with the patient s erythrocytes at low temperatures and that labile serum factors (alexin or complement) caused lysis of the sensitized cells if the temperature was subsequently raised This work represents the greatest single step forward that has been made in the understanding of paroxysmal cold hæmoglobinuria Similar and apparently independent observations were made by Eason (1906)

Donath and Landsteiner s and Eason s observations were quickly confirmed in their essentials by workers in many parts of the world and the diagnostic cold warm procedure for the demonstration of the hæmolysin is still widely referred to as the Donath Landsteiner test The antibody is conveniently referred to as the D–L antibody The technique of the Donath Landsteiner test is described in Chapter 18 (p 493)

Paroxysmal cold hæmoglobinuria has a large literature Reviews (in English) include those of Macalister (1908–9) Mackenzie (1929) and Becker (1948) Nevertheless the disease is a rare one Becker (1949) for instance stated that only one patient had been recognized as suffering from paroxysmal cold hæmoglobinuria out of a total of 130 000 patients admitted to the University of Chicago Clinic in 20 years In Britain the disease is undoubtedly rare and the virtual elimination of congenital syphilis has no doubt contributed to its rarity Of the cases now diagnosed an increasing proportion are likely to be unassociated with syphilis

Clinical Features

A typical attack of paroxysmal cold hæmoglobinuria consists of constitutional symptoms as well as of the passage of hæmoglobin in the urine However each feature can occur without the other The attacks are precipitated by cold the necessary degree of chilling varying from patient to patient sometimes a brief exposure to a minor degree of cold is all that is necessary Usually there is a pause of a few minutes up to several hours before the

CHAPTER 10

ACQUIRED HÆMOLYTIC ANÆMIA (AUTO-ANTIBODY TYPE)

IV PAROXYSMAL COLD HÆMOGLOBINURIA, SYPHILITIC AND NON-SYPHILITIC

THE passage of urine containing hæmoglobin in solution (hæmoglobinuria) as the result of exposure to cold is the essential clinical feature of paroxysmal cold hæmoglobinuria (P C H) In recent years it has become widely accepted that this symptom may develop as the result of at least two apparently distinct disease processes (Stats and Wasserman 1943 Becker 1948 Ferriman Dacie Keele and Fullerton 1951 van Loghem Mendes de Leon Frenkel Tietz and van der Hart 1952) The more familiar typical form of paroxysmal cold hæmoglobinuria is that usually attributed to syphilis the less familiar type occurs in association with Raynaud s phenomena and chronic hæmolytic anæmia of the cold antibody type as already described in Chapter 7 (p 175)

Typical paroxysmal cold hæmoglobinuria will be discussed in the following pages and some evidence will be presented which suggests that whereas syphilis is often an ætiological factor this is not always the case It seems that like acquired hæmolytic anæmia paroxysmal cold hæmoglobinuria cannot be looked upon as a single clear cut disease

History Dressler (1854) has been generally credited with the differentiation from hæmaturia of a case of intermittent chromaturia His ten year old patient may have been a congenital syphilitic In England the disease had been imperfectly described earlier by Elliotson (1832) whose patient had heart disease and cold fits and passed bloody urine whenever the east wind blew It was remarkably accurately described by Harley (1865) Dickinson (1865) and Hassall (1865) All three physicians realized that exposure to cold precipitated their patients attacks and that the urine contained blood pigment but no blood corpuscles Dickinson considered that the disorder was due to an alteration in the blood and likened the urine to that in arsine poisoning! Gotze (1884) and Murri (1885) seem to have been the first to have called atten

steep falls in hæmoglobin and erythrocyte count can occur following a paroxysm Donath and Landsteiner (1925) for instance reported that in one patient the hæmoglobin fell from 85% to 55% as the result of a single paroxysm On recovery from the hæmolytic episode the usual signs of blood regeneration such as a raised reticulocyte count and polychromasia are found

At the height of a paroxysm the patient s plasma typically becomes visibly red the hæmoglobin concentration reaching 100 to 200 mg per 100 ml or even higher In minor paroxysms without hæmoglobinuria a rise in the plasma hæmoglobin concentration can be demonstrated by the benzidine method Following a paroxysm methæmalbumin is found in the plasma for a short while

Leucopenia Interesting leucocyte changes occur during a paroxysm Uchida (1921) found that the lowest counts were reached in five to twenty minutes after exposure to cold the percentage fall being as much as 72% in his most severe case The leucopenic phase lasted from ten minutes to two hours it was followed by a leucocytosis The greatest fall in leucocyte count observed by Bjorn Hansen (1936) was from 10 000 to 2 100 cells per c mm 20 minutes after the commencement of cooling Bjorn Hansen (1936) Totterman (1946) and Jordan Prouty Heinle and Dingle (1952) have shown that the total monocyte and eosinophil counts also fall as does the lymphocyte count to a lesser extent

Erythrophagocytosis Ehrlich (1891) seems to have been the first to have noticed erythrophagocytosis in paroxysmal cold hæmoglobinuria This was seen in blood films made from the finger of a patient which had been chilled whilst the circulation through it was obstructed (Ehrlich test) The phenomenon has been repeatedly observed subsequently (Eason 1907 Meyer and Emmerich 1909 Uchida 1921 etc) Eason reported that neutrophils as well as monocytes acted as erythrophages and that *in vitro* some erythrophagocytosis might be observed using inactivated serum

Recently further studies *in vivo* and *in vitro* have been carried out by Jordan Prouty Heinle and Dingle (1952) They concluded that complement was required for the phagocytosis by neutrophils and monocytes of erythrocytes sensitized by the D L antibody and that the erythrophages were probably removed from the circulation by being trapped in organ capillaries thus at least in part accounting for the neutropenia Bonnin and Schwartz (1954) also found that complement was necessary in the cold phase of sensitization if phagocytosis was to occur subsequently

patient experiences symptoms First pains in the back and legs develop or there may be abdominal cramps or headache The patient may then experience a rigor during which his temperature may rise as high as 104 F The pyrexia may last up to several hours Usually the first specimen of urine passed after the start of the rigor contains hæmoglobin and perhaps methæmoglobin also it may be dark red in colour or almost black As a rule the hæmoglobin disappears within a few hours exceptionally it may persist for a day or more If paroxysms occur frequently significant amounts of hæmosiderin are present in the urine even in the absence of overt hæmoglobinuria

During the attack and for a short time afterwards the patient s spleen may be palpable and on the following day he may be slightly jaundiced

Abortive attacks Hæmoglobinuria may occur in some patients without other symptoms in other patients the constitutional symptoms may occur in the absence of overt hæmoglobinuria — Kaznelson (1921) described such attacks as paroxysmal Kalteikterus Probably however if plasma hæmoglobin concentrations were estimated in such patients hæmoglobinæmia would always be demonstrable as was found by Mackenzie (1929) when he attempted to induce attacks by immersing his patients hands and feet in ice water Transitory albuminuria is also usually found in association with abortive attacks

Vasomotor Phenomena Many authors have reported the association of vasomotor phenomena with attacks of hæmoglobinuria Leaving aside those patients who develop Raynaud s phenomena as a result of intra vasal auto agglutination and whose antibody differs markedly from the D–L hæmolysin (p 250) it seems clear that vascular disturbances may develop in typical paroxysmal cold hæmoglobinuria Both urticarial wheals and cyanosis have been described Harris Lewis and Vaughan (1929) suggested that cold urticaria might be due to an antibody a dermolysin which injured the cells of the skin on exposure to cold They found that the serum of one particular patient when injected intradermally into a non sensitive subject caused the formation of a pruritic erythematous wheal when the site was chilled and then warmed

Hæmatology

Many of the patients suffering from paroxysmal cold hæmoglobinuria become chronically anæmic during cold weather if they have frequent attacks and in seriously affected patients

due to qualitative differences in the antibodies of different patients and to variations in the potency (titre) of the antibodies as well as to differences in technique

The writer has studied the role of complement in the cold phase using serum from two patients In both Case 17 (p 287) and Case 18 (p 288) no lysis developed unless fresh serum was present in the cold phase However it was possible to demonstrate in Case 17 that hæmolytic antibody was adsorbed in the cold phase in the absence of thermolabile constituents of complement even though no hæmolysis occurred subsequently (Table 22)

The patient s serum was inactivated and normal erythrocytes sensitized in it for 30 minutes at 0 C to 2 C (Stage I) The corpuscles were washed twice in a large volume of ice cold saline The button of corpuscles was then placed in the water bath at 37 C and fresh normal serum added No lysis developed after 30 minutes incubation (Stage II) The suspension of cells was then rapidly centrifuged the serum separated and fresh normal corpuscles added to the serum The suspension of fresh cells was then chilled at 0 C for 30 minutes (Stage III) and finally rewarmed (Stage IV) Lysis rapidly developed *This experiment suggests that antibody is adsorbed in the cold phase* in the absence of thermolabile constituents of complement but that it is so rapidly eluted at 37 C under these circumstances that hæmolysis is prevented (see also p 2⌣⌣)

TABLE 22 *A demonstration that the Donath Landsteiner antibody is adsorbed at 0 C in the absence of thermolabile components of complement (for explanation see text above)*

Stage of experiment	Procedure	Hæmolysis
I	Sensitization at 0 C	0
II	Incubation at 37 C (elution of antibody)	0
III	Sensitization of fresh erythrocytes in eluate at 0 C	0
IV	Incubation at 37 C	+

Role of Complement in the Warm Phase All authors are agreed that lysis takes place in the warm phase of the Donath Landsteiner reaction through the agency of complement It has been claimed that sometimes sufficient complement can be adsorbed in the cold phase to bring about lysis when the corpuscles are subsequently warmed (Widal Abrami and Brissaud 1913 Émile Weil and Stieffel 19⌣7)

Most workers e g Siebens Zinkham and Wagley (1948) have not substantiated this claim However Jordan and co workers (1952) stated that there is a reciprocal relationship between the amounts of complement required in the two phases They also claimed that

✓ **Serology**

Cold warm Hæmolysis The feature of the greatest interest in paroxysmal cold hæmoglobinuria is the *Donath Landsteiner hæmolysin* As already mentioned the essential facts about the behaviour *in vitro* of this remarkable antibody were first described by Donath and Landsteiner in 1904 Donath and Landsteiner showed that lysis took place when the erythrocytes of the patient or those from a normal subject were chilled in the serum or plasma of the patient and then subsequently warmed at 37 C They also showed that previous heat inactivation of the patient s serum prevented the onset of lysis The hæmolytic reaction thus appeared to be of the amboceptor complement type and two phases of the cold warm reaction a cold sensitizing phase and a subsequent warm lytic phase were clearly differentiated Subsequent studies have centred around several points of controversy in particular the necessity for complement to be present in the cold phase the thermolability of the lysin the effect that exposure to carbon dioxide or acidification of the serum has on lysis and the highest temperature at which the lysin may be bound

Role of Complement in the Cold Phase Hoover and Stone (1908) reported observations which suggested that the union of hæmolysin and erythrocytes took place in the cold only if complement was present This view was supported by the experiments of Moss (1911) Cooke (1912) and of Dennie and Robertson (1915) and by the work of others This conclusion was disputed by Mackenzie (1929) who nevertheless admitted that more hæmolysis occurred if complement was present during both phases of the reaction

More recently the problem has been re investigated Siebens Zinkham and Wagley (1948) concluded that at least one component of complement was necessary in the cold phase for fixation of the antibody and Jordan Pillemer and Dingle (1951) claimed that it was the C 4 component of complement which was required for fixation of antibody in the cold Van Loghem Mendes de Leon Frenkel Tietz and Hart (1952) in their Case 1 found that although hæmolysis was maximal if fresh human complement was present in the cold phase a certain amount of hæmolysis followed sensitization in heat inactivated serum

The author agrees with the suggestions of Mackenzie (1929) that complement is not necessarily essential for the fixation of the antibody in the cold phase More antibody is bound if complement is present but in some cases at least a variable amount of antibody is bound even if the heat labile fractions of complement are destroyed The discrepancies in the literature seem likely to be

D L hæmolysin its behaviour in respect of temperature and acidification was more like that of the hæmolytic high titre cold antibodies already described (see p 2∪0) Subsequent investigations using sera from presumed cases of paroxysmal cold hæmoglobinuria have mostly failed to confirm Hijmans van den Bergh s observations (Mackenzie 1929) Hannema and Rytma (1922) however stated that they obtained increased lysis in the presence of carbon dioxide and Wagley Zinkham and Siebens (1947) and Siebens Zinkham and Wagley (1948) observed in one of two cases that lysis at 27 C occurred only in the presence of carbon dioxide

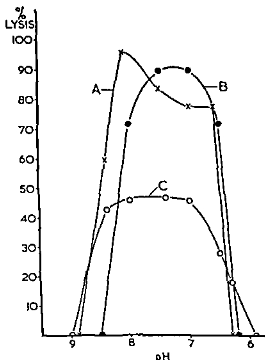


FIG 78 The effect of pH on the hæmolysis of normal erythrocytes by three sera (A B and C) containing hæmolysins of the Donath Landsteiner type

Carbon dioxide probably affects hæmolysis by decreasing pH The author has studied the effect of pH in three cases two being children and one an adult using hydrochloric acid as acidifying agent In each case hæmolysis was almost maximal in unacidified serum (pH 8 0)

hæmolysis occurred in the absence of C 1 and C 3 and that C 2 was alone essential during the warm phase The author found using the very sensitive paroxysmal nocturnal hæmoglobinuria (P N H) erythrocytes and high titre cold antibodies that sufficient complement might be fixed or adsorbed to the corpuscles in the cold phase to produce lysis on subsequent warming There seems no reason therefore why this may not happen when cells are sensitized in highly potent Donath Landsteiner antibodies

Thermolability of the D–L Antibody Yorke and Macfie (1921) Mackenzie (1929) and Siebens Zinkham and Wagley (1948) observed that heating at 56 C to inactivate complement appeared in some cases to destroy the hæmolysin also Mackenzie reported that heating at 45 C for 30 minutes destroyed the hæmolysin in the serum of one of his patients and that in another the hæmolysin was destroyed at a temperature of 47 5 C Yorke and Macfie (1921) stated that the thermolability of one serum appeared to fluctuate from time to time Siebens Zinkham and Wagley (1948) found that whilst one serum they studied was more sensitive than complement to inactivation by heating another serum was less sensitive Jordan Pillemer and Dingle (1951) investigated two sera from this point of view They found that some hæmolytic activity was still demonstrable after the sera had been heated at 62 C for 30 minutes if sufficient complement was subsequently added The hæmolytic activity of the sera studied by the author (Cases 17 and 18) was not affected by heating at 56 C for 30 minutes

These observations lead to the conclusion that although the hæmolysins in the sera of patients with paroxysmal cold hæmoglobinuria vary in their thermolability most antibodies are probably unaffected by a temperature as high as 56 C for 30 minutes Of the thermolabile ones it is probably true that some hæmolytic activity can usually be demonstrated after exposure to quite high temperatures if large amounts of complement are added subsequently

Role of Carbon Dioxide and pH Hijmans van den Bergh (1909a and b) reported that if the whole blood or erythrocytes of a patient suffering from paroxysmal cold hæmoglobinuria were suspended in his serum they underwent hæmolysis at 16° C if exposed to carbon dioxide On the other hand no hæmolysis developed at 16 C without carbon dioxide or at 37 C with or without carbon dioxide This seems to be the first description of an acid lysin in the literature Although in the account of Hijmans van den Bergh there is no mention of agglutination there seems to be little doubt that the antibody was *not* a typical

with the serum from Case 17 maximum lysis did not occur unless the cold phase was prolonged for at least 30 minutes (Fig 79)

Temperature of the Cold Phase The highest temperature at which sensitization takes place probably varies from patient to patient It is likely that the height of the sensitizing temperature is correlated with the severity and frequency of clinical attacks In the literature union of lysin with erythrocytes has been recorded at temperatures as high as 20 C (Grafe 1911) but this is exceptional Usually 10 to 15 C is the maximum Mackenzie (1929) recorded 10 to 12 C in the three cases he studied *In vitro* irrespective of the highest sensitizing temperature maximum lysis is obtained by immersing the cell serum suspensions in water containing crushed ice

Elution of Antibody at 37 C Although lysis usually occurs rapidly at 37 C even within a minute or so with highly sensitized corpuscles it can be shown that antibody is eluted off the corpuscles relatively rapidly at this temperature even though the sensitization has been carried out in the presence of complement

Duplicate suspensions of normal erythrocytes were sensitized in the serum of Case 17 at 2 C for 30 minutes The cells in one tube were then rapidly washed twice in saline chilled at 0 to 2 C whilst the cells in the second tube were washed twice in saline warmed at 37 C Normal serum was added to each cell deposit and both tubes were then incubated at 37 C The cells that had been washed in the cold saline underwent about four times as much lysis as the cells washed in the warm saline The amount of hæmolysis at 37 C is thus the resultant of two processes working in opposite directions—elution of antibody and lysis by complement The apparent elution of incomplete antibody *in vivo* (Jordan Pillemer and Dingle 1952) is referred to on p 282

Other Properties of the Donath Landsteiner Antibody

Agglutination The possibility that the D L antibody causes agglutination as well as lysis has received little attention If sensitization is carried out in the cold using fresh serum containing complement little or no agglutination is usually visible at the end of the cold sensitizing period although the fact that the corpuscles will hæmolyse rapidly on warming shows that they have adsorbed antibody If on the other hand the erythrocytes are suspended in heat inactivated serum agglutination in the cold phase tends to be more intense Nevertheless when such sera are titrated in saline the agglutination titres rarely exceed normal (Stats and Wasserman 1943 Becker 1948) the titres of 160 and 320 respectively recorded by Siebens Zinkham and Wagley (1948) being exceptionally high The cold agglutinin titres (at 2 C) of Cases 17 and 18 of the author were 16 and 32 respectively

only slightly more lysis developing with two sera at the optimum pH of 7 0 to 7 5 (Fig 78) Adsorption of the antibody was inhibited at a pH greater than 8 7 and below 6 2 These observations and the fact that in the great majority of cases described in the literature positive Donath Landsteiner reactions have been observed without the acidification of serum or exposure to carbon dioxide are evidence of a significant difference between the rarely met with Donath Landsteiner antibody and the strongly agglutinating cold antibodies found in cases of acquired hæmolytic anæmia (cf Fig 77 p 251) Nevertheless it is possible that intermediate types of antibody may be developed as the reports of Hijmans van den Bergh (1909) and of Wagley Zinkham and Siebens (1947) already referred to suggest—the sera of their patients causing hæmolysis at 1° C and 27 C respectively when exposed to carbon dioxide although the antibodies were not apparently capable of bringing about strong agglutination On the other hand although agglutinating high titre cold antibodies usually cause hæmolysis only when the serum is suitably acidified exceptions do occur in which acidification tends to inhibit rather than increase hæmolysis (see Table 14 p 252)

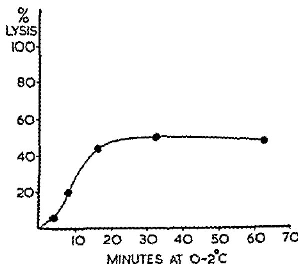


FIG 79 The effect of the duration of sensitization in the cold on hæmolysis by a Donath Landsteiner hæmolysin (Case 17)

Time Necessary for Sensitization in the Cold Phase Yorke and Macfie (1921) observed that maximum sensitization as judged by subsequent hæmolysis took place if erythrocytes were suspended in their patient s serum for relatively short periods they found for instance that more lysis followed chilling for five to seven minutes than for 30 minutes The author and van Loghem and co workers (1952) could not confirm these observations

1950 1953) the sensitization of normal corpuscles in patients sera was shown to be dependent on the presence of thermolabile constituents of serum In both Case 17 and Case 18 there was some evidence that the indirect antiglobulin method was a more sensitive index of sensitization and was positive at a higher temperature than was the hæmolytic test (Table 24 p 289)

Reactions with Trypsinized Normal (T N) Erythrocytes and Paroxysmal Nocturnal Hæmoglobinuria (P N H) Erythrocytes The serum of Case 17 hæmolysed T N erythrocytes slightly more readily than fresh normal untreated corpuscles (hæmolytic titre × 2) and P N H erythrocytes rather more actively (hæmolytic titre × 2 to × 4 that with normal corpuscles) The results with the serum of Case 18 were similar T N and P N H corpuscles were each about twice as sensitive as normal corpuscles

These differences in sensitivity between normal and abnormal erythrocytes are far less than those observed with the strongly agglutinating cold antibodies which only weakly hæmolyse normal corpuscles (see p 253)

Specificity of the Antibodies It has generally been assumed that the Donath Landsteiner hæmolysin is a non specific antibody However in at least one patient it has been claimed that the antibody was strictly auto specific (van Loghem *et al* 1952) The serum of Case 18 was tested with erythrocytes of Groups O A_1 A_2 B and A_1B respectively The sensitivity of the cells to hæmolysis was about the same if anything the most sensitive cells seemed to be those of groups A_1 and A_2

Serum Complement It has been known for a long time that the serum complement concentration may be abnormally low in paroxysmal cold hæmoglobinuria (Meyer and Emmerich 1909) and that following repeated attacks of hæmolysis complement may not be demonstrable The serum may even be anti complementary (Mackenzie 1929) These possibilities have to be taken into account in the investigation of supposed cases of the malady (see p 493) There seems to be no information as to which components of complement are deficient

Correlation of Antibody Titres with Clinical Signs of Hæmolysis Few detailed studies of antibody titres and of the thermal range of the antibody in relation to the clinical course of the disease have been published Kumagai and Namba (1927) however published some interesting data according to these workers a hæmolytic titre greater than 8 was associated with spontaneous attacks of hæmoglobinuria at a titre of 4 hæmoglobinuria could be induced by artificial chilling at a titre of

It seems likely however, that the D L antibody causes agglutination as well as hæmolysis (in addition to causing sensitization to antiglobulin serum) it would perhaps be surprising if it did not In two patients (one of them Case 17) studied by the author agglutination of normal corpuscles was noted to persist for as long as 2 hours at 37 C in partially hæmolysed cell serum suspensions that had been chilled before being warmed In Case 18 too the ability of the patient s serum to agglutinate corpuscles at 18 C and to sensitize them to antiglobulin serum diminished in a parallel manner as the patient recovered (Table 24)

Sensitization to Antiglobulin Serum Fisher (1950) *Jordan Pillemer and Dingle (1951)* Peterson and Walford (1952) and van Loghem and co workers (1952) have all reported that erythrocytes sensitized with the D L antibody *in vitro* give positive antiglobulin reactions

Jordan Pillemer and Dingle (1951) also reported that the erythrocytes of their patients gave strongly positive direct antiglobulin reactions at the time of hæmolytic attacks produced by chilling The reaction however became negative shortly after the attacks Their observations suggest that the antibody they were studying sensitized corpuscles to antiglobulin serum at a relatively high temperature *in vivo* and that the sensitization was slowly reversible at body temperature Both Jordan Pillemer and Dingle (1951) and van Loghem and co workers (1952) showed that as with other types of cold antibodies the antiglobulin reaction was not inhibited in high concentrations of antiglobulin serum

The indirect antiglobulin reaction was studied in some detail by Jordan Pillemer and Dingle (1951) In two patients the antibody titres as measured by hæmolysis and by agglutination using a constant amount of antiglobulin serum were identical Jordan and his co workers concluded that the hæmolytic antibody and the sensitizing antibody were probably the same substance By fractionation of the serum of one case they obtained evidence which they took to indicate that the antibody was a γ globulin

The author has made observations on the antiglobulin reactions in three patients The direct test was repeatedly negative in *Case 17 who was always symptom free when tested* but was positive at the height of the hæmolytic episode in Case 18 (Fig 81) and in one other patient in an active phase In each case maximum (direct or indirect) reactions were obtained in strong concentrations of antiglobulin serum and in each case too agglutination persisted after the addition of relatively large amounts of normal human γ globulin to the antiglobulin serum (Tables 10 and 11) It was concluded therefore *that the sensitizing antibodies were probably* not normal γ globulins As with other cold antibodies (Dacie

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2 to 4 albuminuria could be induced by chilling but with anti bodies weaker than this no changes of any kind could be produced

The child described as Case 18 has been followed in some detail In this (non syphilitic) case spontaneous cure was closely correlated with diminution in the antibody concentration and in the temperature at which it would produce sensitization (Fig 80 and Table 24)

Summary

The main features of the *Donath Landsteiner antibody* are as follows The antibody is remarkable in that fixation on to erythrocytes takes place only in the cold usually at temperatures below 15 C Hæmolysis is brought about in the warm phase by the *action of complement Complement is also more or less essential* for the effective fixation of the antibody in the cold phase however it may act in this phase not so much by bringing about the adsorption of the antibody as by preventing the antibody *from being rapidly eluted on subsequent warming The* D L antibody is remarkable amongst anti erythrocyte antibodies in that it so readily causes lysis of normal human erythrocytes instead of only or predominantly causing agglutination Although *lysis is the most obvious effect the D L antibody* causes agglutination as well as strong sensitization to antiglobulin serum The optimum pH for hæmolysis is in the region of pH 7 0 to 8 0

Technique of the Donath Landsteiner Reaction This is described in detail in Chapter 18 (p 493)

Ætiology

Relation to Syphilis As already mentioned syphilis was *suspected to be an important ætiological factor in the* nineteenth century by some of the earlier writers on paroxysmal cold hæmoglobinuria (Gotze 1884 Murri 1885) Murri for instance reviewed 36 cases and thought that there was evidence for syphilis in fifteen of them The frequency of the disease in congenital syphilitic children and the occasional presence of lesions of acquired syphilis in adult patients all seemed to bear this out The improvement in diagnosis resulting from the introduction of the Wassermann reaction (W R) likewise appeared to furnish further support for this contention

Donath and Landsteiner (1925) in a review of 99 patients considered that there was evidence of syphilis in 95 in 81 patients the W R was positive whilst in 24 of them there was clinical evidence of syphilis

Moreover it seems probable that in the past at least the sera of an appreciable proportion of patients with late syphilis contained the D L hæmolysin even if the patients had not suffered from clinical paroxysmal cold hæmoglobinuria Kumagai and Namba (1927) for instance found that a cold autohæmolysin was present in the sera of seven out of 35 such patients and that Ehrlich s finger test (Ehrlich 1891) was also positive Mackenzie (1929) concluded (1) that paroxysmal cold hæmoglobinuria was usually and perhaps always a manifestation of syphilis and (2) that a small percentage of patients with late syphilis have the latent form of paroxysmal hemoglobinuria He also made the point that paroxysmal cold hæmoglobinuria appeared only in the quiescent stage of late syphilis

Becker (1948) also concluded that syphilis was the cause of paroxysmal cold hæmoglobinuria He reviewed 37 reports in the literature published since 1930 and added a case of his own Ten of the patients were children the parents of eight of the children were investigated and clinical or serological evidence of syphilis established in each case In none of the 37 patients was there clinical evidence of active syphilis at the time of the development of the hæmoglobinuria although some gave a history of infection years previously On the other hand in eleven of the patients cited positive serological reactions were the only signs of the syphilis their personal histories and clinical examination being negative In eight of the patients anti syphilitic therapy was associated with amelioration or disappearance of the hæmoglobinuria Whether or not this was the consequence of the treatment cannot be determined for very little seems to be known of the course of the disease in patients not receiving anti syphilitic treatment

Mackenzie (1929) stated that clinical attacks may last for many years However this is clearly not always so Browning and Watson (1912–13) for instance reported the case of a boy who suffered from an attack of brief duration but had no further attacks during the two years he was under observation At the end of this time the Fason phenomenon (D L test) was still positive As the course of the disease is uncertain it seems unsafe to accept an apparently favourable response to anti syphilitic therapy as evidence for a syphilitic ætiology for paroxysmal cold hæmoglobinuria

Burmeister s (1921) conclusions differed from those of Mackenzie and of Becker Of 207 cases reported in the literature he considered that there were indications of syphilis in only 79 (38%) although the Wassermann reaction was positive in 95% of the cases in which it was carried out Contrary to other observers (see Mackenzie 1929) Burmeister found that when he absorbed the hæmolysin with erythrocytes in the cold the Wassermann reacting substance was also absorbed and that when the hæmolysin was dissociated from the sensitized corpuscles by warming the eluate gave a positive Wassermann reaction Burmeister concluded that some cases of paroxysmal cold hæmoglobinuria occurred in the absence of syphilis even though the Wassermann reaction was positive

The role of syphilis in paroxysmal cold hæmoglobinuria is made more questionable by the knowledge that positive Wassermann and Kahn reactions are not uncommon in acquired hæmolytic

anæmia of the auto antibody type (see p 195) in these cases the positive reactions seem to be the result of the presence of abnormal serum constituents quite apart from the anti erythrocyte antibodies the stimulus for formation being generally unknown but almost certainly quite unconnected with syphilis It seems probable as was suggested by Burmeister in 1921 that some of the recorded cases of paroxysmal cold hæmoglobinuria in which positive serological reactions were the only signs of syphilis really belonged to the non syphilitic group as indeed do those patients in whom the serological reactions and clinical and family studies are entirely negative (Sweetnam Murphy and Woodcock 1952 and Case 17) Kaiser and Bradford (1929) and Gasser (1951) have in fact described patients in whom the Wassermann and Donath Landsteiner reactions were both positive at the height of the attacks only to become negative on the patients recovery

It seems more logical therefore to consider paroxysmal cold hæmoglobinuria as a syndrome of varying ætiology the connecting link between the different types being the formation of a cold antibody of more or less uniform characteristics At least three clinical types may be distinguished (Table 23) the non syphilitic types being probably closely allied ætiologically to acquired hæmolytic anæmia of the auto immune type but differing in the peculiar type of antibody which is formed

TABLE 23 *A Tentative Classification of the Paroxysmal Cold Hæmoglobinurias*

Paroxysmal cold hæmoglobinuria	(1) Syphilitic chronic type	
	(2) Non syphilitic types	(a) Acute transient type Idiopathic or ? secondary (Case 18)
		(b) Chronic Idiopathic type (Case 17)

Treatment of Paroxysmal Cold Hæmoglobinuria

It seems logical to treat patients in whom there is definite evidence of syphilis with anti syphilitic drugs bearing in mind that positive serological tests alone without any other evidence of syphilis may be unreliable guides to the ætiology of the disease

In non syphilitic cases no specific treatment is available however it would be worth while giving cortisone or A C T H a trial in patients in whom attacks of hæmolysis are frequent and

serious In all patients exposure to cold and damp should be avoided as far as is possible

Case Report Paroxysmal Cold Hæmoglobinuria not Associated with Syphilis

Case 17 The patient (D O) was a young man aged 18 years In December 1949 whilst undergoing military training in very cold conditions he passed a specimen of urine which was dark red in colour he had been out on an exercise at night and had become very cold as the result of lying on wet grass The hæmoglobinuria lasted for about half the following day He had never had this symptom before nor did it recur He had always enjoyed excellent health and this single episode of hæmoglobinuria was not associated with any constitutional symptoms He was first investigated by the writer in January 1950 and has been seen subsequently at intervals for three and a half years He has felt perfectly fit during the whole time and has not had any further attacks of hæmoglobinuria He has received no treatment

Physical Examination He was found to be a well built healthy looking young man Examination revealed no abnormalities in particular there were no signs suggestive of congenital or acquired syphilis

Laboratory Investigations His blood count was normal and the blood Wassermann and Kahn reactions negative His C S F was also normal the C S F Wassermann and colloidal gold reactions were also negative His parents and a younger sister were alive and well their Wassermann and Kahn reactions were negative

Serology His blood has been examined on nine occasions between January 1950 and June 1953 On each occasion the direct antiglobulin reaction was negative On each occasion however blood allowed to clot at 2 C for 30 minutes and then subsequently warmed at 37 C underwent spontaneous hæmolysis Blood allowed to clot at room temperature (15 to 20 C) and at 37 C did not hæmolyse The Donath Landsteiner reaction using unacidified patient s serum and normal corpuscles was consistently positive although the antibody titre using normal corpuscles has decreased from 16 in March 1950 to 2 in June 1953 The upper thermal limit for sensitization did not exceed 10 C although sensitization to antiglobulin serum took place at 15 C The cold agglutination titre at 2 C has not been greater than 16

Complement was required for sensitization in the cold phase while the antibody itself withstood heating at 56 C for 30 minutes The patient s serum complement concentration was within the normal range The effect of time on sensitization in the cold phase the effect of pH the indirect antiglobulin reaction and elution of the antibody in the warm phase and the reactions with trypsinized normal corpuscles and P N H erythrocytes have been referred to on pp 279 to 283

Summary A case of paroxysmal cold hæmoglobinuria of unknown origin apparently *not* due to syphilis A cold hæmolysin of the D L type was present its activity *in vitro* slowly declined but the antibody was still present three and a half years after the initial and only attack of hæmoglobinuria

Case Report Acute Paroxysmal Cold Hæmoglobinuria (?) Following Measles Spontaneous Recovery

Case 18 The patient (D F) was a little girl aged 3 years Nine days before admission into hospital she developed an apparently typical attack of measles The disease took a normal course until the day before admission when she had a rigor No abnormal physical signs were found at this time except those of a slight upper respiratory tract infection However the following day she passed dark red brown urine and was brought into hospital in consequence She had not been given any sulphonamide drugs during her illness

Physical Examination On admission on January 5th 19-3 she was found to be a rather ill looking child The remains of a measles rash could be seen on her body No other abnormal physical signs were elicited however and neither her spleen nor liver was palpable Her temperature was 100 F Her urine was normal except for traces of urobilin

The following day she was noticed to be slightly jaundiced There were no other abnormal signs and her urine was again normal except for excess of urobilin On the second day after admission she was

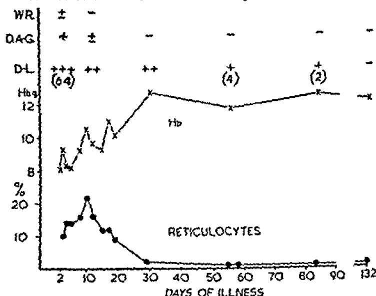


FIG 80 Serological studies during the recovery of a patient from acute cold hæmoglobinuria (Case 18) W R = Wassermann reaction D.A G = direct antiglobulin test D L = Donath Landsteiner test (the figures in parentheses refer to the titre of the cold hæmolysin)

taken at 2 p m into the bathroom where she may have become chilled for at 5 30 p m 7 p m and at 10 p m the urine she passed contained obvious free hæmoglobin By the following morning however the urine had become normal once more Her liver was then found to be

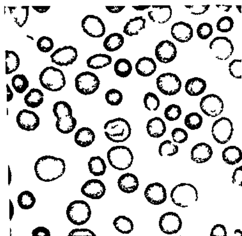


FIG 81 I hotom c ograph of a l lood film of a pati nt uffering from acute col l hæmoglol nur a (C1 e 18) × 700

slightly enlarged but her spleen could not be felt. Thereafter she made an uninterrupted recovery and no further attacks of hæmoglobinuria have occurred.

Laboratory Findings. On admission her hæmoglobin was found to be 8.1 g per 100 ml, the M.C.V. was 100 c µ and the total leucocyte count 12,000 cells per c mm with 65% neutrophils. Stained peripheral blood films showed considerable anisocytosis, polychromasia and spherocytosis (Fig. 81) and also very occasional normoblasts. Osmotic fragility was increased: initial lysis 0.60% NaCl, complete lysis 0.20% NaCl, with an M.C.F. of 0.40% NaCl. The serum bilirubin was 1.2 mg per 100 ml. The plasma proteins were normal: albumin 4.6 g per 100 ml, globulin 2.7 g per 100 ml. On admission the Wassermann reaction was doubtful and the Kahn test negative; 8 days later the results of both tests were negative. The Wassermann and Kahn reactions of both the child's parents were negative.

The changes in hæmatological findings during her recovery are illustrated in Fig. 80.

Serology. The child was group AB Rh positive. The direct antiglobulin test was positive on admission; the reaction became gradually less strong and within a month it was negative. When venous blood was obtained from the child soon after admission it was immediately obvious that a hæmolysin of high thermal activity and potency was

TABLE 24. *Serial observations on the reactions* in vitro *between an antibody of the Donath-Landsteiner type and normal erythrocytes during the recovery of Case 18 from an acute but transient episode of paroxysmal cold hæmoglobinuria. The antiglobulin reactions were carried out on any corpuscles remaining unhæmolysed after washing them in several changes of saline warmed to 37° C.*

Day of illness	Temperature of sensitization	Hæmolysis (30 min. at 37° C.)	Agglutination (at temperature of sensitization)	Antiglobulin reaction
+ 3	2 C	+++	++	++++
	18 C	++	+	++++
	25 C	+	±	+++
	30 C	0	trace	+
	37 C	0	0	0
+ 55	2 C	++	+	++++
	18 C	0	0	0
	37 C	0	0	0
+ 83	2 C	+±	++	++++
	18 C	0	0	0
	37 C	0	0	0
+ 14	2 C	0	0	+
	18 C	0	0	0
	37 C	0	0	0

present for only if blood was collected into a warmed syringe was it possible to obtain serum or plasma free from hæmolysis

In vitro normal corpuscles were hæmolysed by the patient's serum when sensitizations were carried out at temperatures up to 25 C The decline in antibody activity which paralleled clinical recovery is shown in Table 24 and Fig 80 Complement was required for fixation of the antibody in the cold phase but the antibody itself withstood heating at 56 C for 30 minutes The effect of pH is illustrated in Fig 78(A) The cold agglutinin titre was never greater than 32 The indirect antiglobulin reaction which seemed to be a more sensitive way of demonstrating antibody action than was hæmolysis and the antibody's capacity to cause agglutination are referred to on p 282 (see also Table 24) and the reactions with trypsinized normal erythrocytes and P N H corpuscles on p 283

Summary A case of transient acute paroxysmal cold hæmoglobinuria developing possibly as a sequel to measles A potent cold hæmolysin of the D L type was present This was active *in vitro* at a relatively high temperature at least up to 25 C Its disappearance coincided with the complete recovery of the patient

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CHAPTER 11

ACQUIRED HÆMOLYTIC ANÆMIA (AUTO ANTIBODY TYPE)

V ÆTIOLOGY AND PATHOGENESIS

Ætiology

THE cause or causes of the development of abnormal auto antibodies in acquired hæmolytic anæmia are not clearly understood It is possible that there are two main mechanisms (1) an alteration in the patient s erythrocytes which has the effect of making them seem foreign to his own antibody forming mechanism and thus antigenic and (2) the development of anti erythrocyte antibodies in the course of the formation of abnormal plasma proteins by the patient the primary abnormality being an unusual reaction on the part of his antibody forming tissues rather than a change localized in his erythrocytes

Altered Erythrocyte Antigenicity in Acquired Hæmolytic Anæmia

It certainly seems reasonable that a chemical such as a sulphonamide drug might so alter the surface of the erythrocytes as to render them antigenic or that a combination of the drug and a component of the erythrocyte surface might function as an antigen in the same sort of way that many chemicals are known to combine with proteins and to impart to them greater or lesser degrees of foreignness (see Wright 1953) Similarly it is conceivable that much the same sort of alteration to the erythrocyte surfaces might result from damage by viral or bacterial enzymes or other products of their metabolism and that antibodies might be formed in response to the presence of these altered cells However there seems to be as yet no unequivocal evidence in favour of this type of hypothesis in relation to hæmolytic anæmia in man Some arguments can be advanced against it For instance if the antibodies are formed as the result of an antigenic alteration in the patient s own erythrocytes it is difficult to see why *normal* erythrocytes should be acted upon so readily *in vitro* and why *normal* erythrocytes should be destroyed so rapidly *in vivo* when transfused to patients It is also hard to understand why in some cases antibodies such as anti e which act on normal

surface antigens should be produced Again if it is argued that the 'non specific type of antibody is developed as the result of the exposure of deep antigens it is difficult to see why normal corpuscles should be agglutinated or sensitized by such antibodies It should perhaps be added however that Stats and Wasserman (1952) considered that the idea that antibodies developed against damaged or altered corpuscles should have marked effects on normal cells was not unreasonable In relation to this point it will be recalled that Davidsohn and Oyamada (1953) claimed that the antibodies certain patients developed were in fact strictly auto specific As already mentioned on p 233 this has not been the present author s experience Were this so it should be possible to transfuse such patients satisfactorily with normal erythrocytes a state of affairs which clinical experience suggests is rare to say the least in acquired hæmolytic anæmia

Dodd and co workers (1953) reported observations which might be taken as indicating that the erythrocytes from patients with acquired hæmolytic anæmia have abnormal surfaces Rabbits were immunized with normal human erythrocytes and trypsinized human erythrocytes respectively It was then found that both types of rabbit sera absorbed with normal human corpuscles still reacted with the trypsinized corpuscles suggesting that as the result of trypsinization some previously hidden antigen had been revealed Especially interesting was the finding that the erythrocytes of fifteen out of nineteen patients with acquired hæmolytic anæmia were also agglutinated by the rabbit sera previously absorbed with normal corpuscles Whether this means that the cells which reacted positively had modified surface antigens or whether agglutination was merely a sign of damage to the cells surfaces which might have arisen from adsorption of antibodies is not clear it i interesting to note that positive results were also obtained in three out of thirteen patients with hereditary spherocytosis

There is some evidence in animals that manipulation or modification of their erythrocytes *in vitro* will exceptionally make them auto antigenic

Wagley and Castle (1949) found that one out of four dogs injected with various antigens composed of autologous erythrocytes and streptococcal toxin or staphylococcal culture filtrate or with Freund antigen (erythrocytes lanolin tubercle bacilli and pig serum) respectively developed a transient positive direct antiglobulin reaction The dog did not however show any signs of hæmolysis

Liu and Evans (195_) observing that a patient developed a positive direct antiglobulin test after an intraperitoneal hæmorrhage inoculated twelve rabbits intraperitoneally for four weeks with their own blood Four of the animals developed positive direct antiglobulin tests but none became anæmic Erythrocyte stroma exposed to a streptococcal filtrate was injected into another series of experimental animals but

none of the rabbits showed any signs of autosensitization Liu and Evans (1952) suggested that the blood injected intraperitoneally being exposed to tissue enzymes and unsaturated fatty acids in lymph might conceivably have undergone surface changes and been rendered antigenic thereby They referred to the clinical impression that in the background of patients suffering from acquired hæmolytic anæmia there was an unusual incidence of infection trauma and medication

The Role of Viruses

The effects of virus action on erythrocytes have been recently reviewed by Briody (1952) Whether or not viruses play a direct part in the genesis of hæmolytic anæmia by their effect on the surfaces of erythrocytes has yet to be proved Moolten and Clark (1952a and b) and Moolten and co workers (1953) described the isolation of the virus of Newcastle disease (N D V) from the blood of patients suffering from acquired hæmolytic anæmia N D V primarily affects birds but it has been detected in man and other species of mammals Autohæmagglutination was a marked feature in Moolten and Clark s (1952a) first case and was attributed by them to adsorption of virus on the erythrocyte surfaces and not to the presence of abnormal auto agglutinins Subsequently N D V the virus of herpes simplex and other unidentified viruses were isolated from other patients suffering from various types of hæmolytic anæmia (Moolten and Clark 1952b Moolten *et al* 1953) Up till now this work does not seem to have been confirmed in fact what evidence there is seems to be against the hypothesis that N D V is a common ætiological factor Morgan (1952) searched for viruses in the blood of six patients and the spleens of three patients with acquired hæmolytic anæmia but none was isolated Eyquem and Dausset (1952) studied the sera of 129 patients suffering from various types of anæmia Seven sera inhibited the agglutination of erythrocytes by N D V three of them contained a powerful inhibitor but only one of these was from a patient with acquired hæmolytic anæmia the other two were from patients suffering from hereditary spherocytosis and paroxysmal nocturnal hæmoglobinuria respectively

Betke Richarz Schubothe *and Vivell (1953) have recently* described a child who developed an acute hæmolytic anæmia of the auto immune type with a positive antiglobulin test in whom infection with Coxsackie virus A was demonstrated The virus was isolated from the fæces at the time of the infection and neutralizing antibodies were demonstrated in the patient s serum during convalescence

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In the type of hæmolytic anæmia which appears to be related to infection a peculiar proneness of the patient to develop anti bodies is probably an essential factor in the genesis of the antibody response There seems no doubt that certain people form iso antibodies with extraordinary ease following transfusions (Callender and Race 1946 Malone and Cowan 1950 Collins Sanger Allen and Race 1950 Waller and Race 1951) and it seems likely that other things being equal these are the people who develop hæmolytic anæmia of the auto antibody type—certainly some sufferers from acquired hæmolytic anæmia readily form iso antibodies if transfused (p 195)

Rantz (1953) has recently made an observation which also seems to suggest that certain people possess an unusual immunological hyperactivity He found that normal sera contain a factor which in association with complement hæmolyses human erythrocytes treated with various bacterial products In patients suffering from collagen disease or from acquired hæmolytic anæmia this serum factor was present in unusually high concentrations

Possible Genetical Factors Whether or not susceptibility to acquired hæmolytic anæmia and abnormal antibody formation is genetically determined is uncertain As previously mentioned on p 167 there is at least one report in the literature of the develop ment of hæmolytic anæmia of auto antibody type in a mother and in her daughter in two other relatives of this family there was pronounced thrombocytopenia and it is possible that in these cases the tendency to develop antibodies was inherited On the other hand as described on p 197 one of the author s patients (Case 9) had an identical twin sister who seven years after the onset of her sister s illness had not yet shown any signs of developing hæmolytic anæmia

It has been claimed that there is a relationship between the ABO blood groups and the ability to secrete blood group antigens in saliva and susceptibility to acquired hæmolytic anæmia Hunt and Lucia (1953) found that of 27 patients suffering from acquired hæmolytic anæmia 78 were group O 7 % group A and 15% group B and con cluded from this that group O subjects were more susceptible The author s data do not support this contention of 28 patients suffering from acquired hæmolytic anæmia of the warm antibody type nine were group O fourteen group A four group B and one group AB a distribution which does not differ significantly from that of the popula tion as a whole in Britain In seven of the group A patients the sub group was known three were group A and four group A The *Lewis* groups were known in four cases three patients were of the genotypes Le /Le^b or Le^b/Le^b and one was *Lewis* negative (Le(a b)) None of them was a non secretor (Le^a/Le^a)

Betke and co workers in discussing the possible connection between the virus infection and hæmolysis concluded that the hæmolysis *might have resulted either from the virus causing* some alteration of the erythrocyte surface rendering it antigenic or to non specific stimulation of the antibody forming tissues of the body They considered that it was unlikely that the auto agglutination was caused by the mere presence of the virus adsorbed on the erythrocyte surface for this would not *provide an explanation for the positive antiglobulin* test They also considered that it was unlikely that antiviral antibodies were bound to the cell by virus adsorbed on the cell surface for although the positive antiglobulin test could be explained in this way the antiviral antibodies did not in fact appear in the child s serum until after the hæmolytic episode had subsided

Abnormal Protein Formation in Acquired Hæmolytic Anæmia

The alternative hypothesis that the antibodies acting on the patient s erythrocytes are but part of an abnormal formation of plasma protein deserves serious consideration Certainly it is possible to present some arguments in its favour For instance the auto antibodies of acquired hæmolytic anæmia are often accompanied by increases in the total amounts of serum globulin and alterations in the electrophoretic patterns of the serum proteins as well as by serum components which give positive Wassermann and Kahn reactions (see p 195) In iso immunization against human blood group antigens on the other hand the *antibodies developed are strictly specific for the antigen concerned* The association with thrombocytopenia (possibly due to antibodies active against platelets) and the not infrequent incidence of hæmolytic anæmia in association with disseminated lupus erythematosus may also be quoted as illustrations of the broad nature of the immunological responses of patients who develop acquired hæmolytic anæmia

Abnormal protein (including erythrocyte antibody protein) can probably arise as the result of several different stimuli In susceptible subjects certain infective agents may act as *hetero* genetic stimuli for example the viruses of virus pneumonia and infectious mononucleosis hæmolytic anæmia being a not uncommon complication of the former Infection with syphilis too very occasionally results in the development of hæmolytic antibodies (see paroxysmal cold hæmoglobinuria Chapter 10) It is reasonable to suppose that infection with other as yet unknown possibly viral agents may be the cause of some of the cases of acquired hæmolytic anæmia at present considered to be idiopathic in origin as in the patient described by Betke and co workers (1953) mentioned above

anæmia it is unfortunately less easy to visualize what exactly is taking place *in vivo* on the basis of the reactions of the antibodies *in vitro*. That the patient's erythrocytes have adsorbed abnormal protein on their surfaces is readily demonstrated by, for instance, the antiglobulin reaction. The problem yet to be solved is how the sensitization shortens the cells' expectation of life. The subject has been recently well reviewed by Wasastjerna (1953). The work of Swisher, O'Brien and Young (1953) on experimental hæmolytic anæmia in dogs has also been of particular importance in emphasizing the different ways in which antibodies of different serological behaviour *in vitro* bring about hæmolysis *in vivo*.

Mechanism of Erythrocyte Destruction in Acquired Hæmolytic Anæmia of the Warm antibody Type

In only a small proportion of patients suffering from acquired hæmolytic anæmia of the warm antibody type can the auto-antibodies be shown to have the property of causing hæmolysis *in vitro*, and in the few patients in which lysis can be demonstrated it can only be done as a rule by using enzyme-treated and/or P.N.H. erythrocytes. It is certain nevertheless that rapid hæmolysis *in vivo* can take place even though it is quite impossible (by present methods) to demonstrate that the auto-antibodies have any hæmolytic power at all *in vitro*. Two of the patients the author has studied (e.g. Cases 11 and 12) have in fact died as the result of fulminating hæmolytic episodes although all laboratory tests for hæmolysins were negative. In these patients, as in many non-fatal cases, it seems that blood destruction *in vivo* must be brought about by other means than by direct lysis through the agency of antibody and complement. Three additional phenomena of antibody action are probably of particular importance in this respect; these phenomena are *autohæmagglutination*, *spherocytosis* and *erythrophagocytosis*.

Autohæmagglutination. It is known that erythrocytes which have adsorbed antibody protein on their surfaces tend to undergo auto-agglutination when left undisturbed in their own plasma *in vitro* at 37° C. There is some evidence, too, that this also occurs *in vivo* both in man and in animals in which experimental hæmolytic anæmia has been produced (Bessis and Freixa, 1947; Wasastjerna, 1948, 1953; Wasastjerna, Dameshek and Komninos, 1954). It is possible that this type of auto-agglutination due to the corpuscles being sensitized by incomplete antibodies may be of particular importance in internal organs such as the spleen through which the circulation is slow (Wagley, Shen

The association of acquired hæmolytic anæmia of the auto antibody type with other disease processes is undoubtedly significant from the point of view of the ætiology of the disease As already referred to hæmolytic anæmia of the auto antibody type is not uncommon in association with chronic lymphatic leukæmia and in cases of reticulosis and reticulosarcoma In such patients there is an abnormal proliferation of cells the normal counter parts of which are probably concerned in the formation of anti bodies It may be that the same stimulus that causes the cellular proliferation causes the development of antibodies or alterna tively that the antibodies are simply products derived from the rapidly growing cells Aubert and Brendemoen (1949) and Wiener Gordon and Gallop (1953) for instance recorded the extraction of high titre cold agglutinins from lymphosarcoma tissue It is interesting in this connection to note that plasma cell proliferation is in myelomatosis is not typically associated with the formation of antibodies active against erythrocytes

Any discussion on *Ætiology* is of necessity incomplete and difficult to summarize However although the development of the abnormal antibodies of acquired hæmolytic anæmia is in the main ill understood certain associations appear significant The disorder probably affects people with an unusual propensity for developing antibodies there may be an overlap with the collagen diseases heterogenetic stimuli such as the virus of virus pneu monia are certainly important and neoplastic hyperplasia of the cells probably normally responsible for antibody formation some times seems to lead to auto antibody formation The attractive hypothesis that viruses may have a direct effect on the patients erythrocytes rendering them antigenic is as yet unproven

PATHOGENESIS OF ACQUIRED HÆMOLYTIC ANÆMIA

It can hardly be doubted that the auto antibodies of acquired hæmolytic anæmia are an important cause of the excessive rate of blood destruction *in vivo* However it is not clear whether they are the sole cause of the hæmolysis nor are the exact mechanisms *by which the antibodies bring about hæmolysis fully understood* In certain instances such as in paroxysmal cold hæmoglobinuria the abnormal antibody can be shown to cause dramatic and clear cut hæmolysis *in vitro* and it seems reasonable to suppose that what happens *in vivo* is being reproduced by the laboratory experiment However in most cases of acquired hæmolytic

given (Banti 1913 Dameshek and Schwartz 1938 Tigertt Duncan and Hight 1940 Bessis and Freixa 1947 Baumgartner 1947 Wasastjerna 1948 Young Ervin and Yuile 1949 Lehmann Rothe and Nitsch 1952) Spherocytosis and increased fragility of the recipient s corpuscles also result from the transfusion of group O plasma containing immune anti A to human group A subjects (Ebert and Emerson 1946 Ervin and Young 1950 Ervin Christian and Young 1950) It is however a remarkable fact that spherocytosis and increased osmotic fragility are *not* readily produced by the action of immune sera on erythrocytes *in vitro* (Banti 1913 Wasastjerna 1948 Castle Ham and Shen 1950)

Muir and McNee (1911–12) Banti (1913) Wasastjerna (1948 1953) and others have all remarked on another notable discrepancy between the effects of antisera on erythrocytes *in vitro* and *in vivo* namely that a given dose of immune serum regularly destroys many more corpuscles *in vivo* than it seems to be capable of hæmolysing *in vitro* Banti (1913) Dameshek and Schwartz (1938) and Wasastjerna (1948) also observed that the effects of an injection of immune serum were not maximal shortly after the time of the injection but developed progressively Wasastjerna for instance reported that the erythrocyte counts of guinea pigs did not reach their lowest levels until three to four days after intracardiac injection of the antibody Dameshek and Schwartz (1938) and Wasastjerna (1948) found that spherocytosis also increased progressively and that this change preceded the fall in erythrocyte count

Recently observations have been made which at first sight seemed to offer some explanation for the potentiation *in vivo* of the effects of anti-erythrocyte sera and their prolonged action Muratore Cervellera and Gardai (1953) inoculated guinea pigs with small doses of hæmolytic sera obtained from rabbits immunized with guinea pig erythrocytes Nothing happened for three days then a rapid hæmolytic anæmia supervened Erythrocyte osmotic fragility became markedly increased and auto-agglutination was noticeable At the time of the hæmolytic crisis incomplete auto-antibodies were present The direct anti globulin test (using the serum of a rabbit immunized with guinea pig serum protein) became positive in four out of six animals and the indirect test positive in all six animals Muratore and co-workers concluded that the delayed hæmolytic anæmia was due to the development of incomplete auto-antibodies which supplemented the effect of the small amount of immune serum injected

It is possible that in the experiment of Muratore Cervellera and Gardai the rabbit serum acted as a heterogenetic stimulus for auto-immunization That this may be the correct explanation has been shown by some very interesting experiments carried out by Samaille

Gardner and Castle 1948) In the spleen particularly auto agglutination might be expected to result in an almost complete *arrest of the circulation which would provide an opportunity for* the erythrocytes to be still further sensitized and to undergo firmer auto agglutination because of the close proximity of the corpuscles to *potentially antibody forming lympho reticular* tissue Wagley and his co workers (1948) in support of this conception reported that erythrocytes obtained from spleens removed at operation from three patients with acquired hæmolytic anæmia were more strongly agglutinated by antiglobulin sera than were corpuscles obtained from the peripheral circulation

The type of agglutination referred to in the preceding paragraph is that brought about by incomplete antibodies in those rarer cases in which powerful in saline agglutinating auto antibodies are formed agglutination is probably an all important factor in *bringing about erythrocyte destruction in vivo* (e g Case 12)

The importance of auto agglutination and stagnation of blood as a cause of corpuscular destruction and of increased fragility has been further emphasized by Castle Ham and Shen (1950) In an important paper Castle and his colleagues presented arguments in favour of the concept that auto agglutination by causing stagnation or arrest of the circulation and subsequent tissue ischæmia leads to the release of substances from the autolysing tissues which have a local damaging effect on the impacted erythrocytes They were able to show in support of their hypothesis that erythrocytes incubated with autolysing tissues developed increased osmotic and mechanical fragility and that the presence of weakly agglutinated corpuscles caused a marked *retardation in the blood flow through organs* experimentally perfused It is interesting to recall in connection with this concept the frequency of liver necrosis in fatal cases of acquired hæmolytic anæmia in man (see p 180)

Spherocytosis As mentioned in Chapter 1 spherocytosis results from an irreversible contraction of the erythrocyte surface which may be brought about either by an inherited defect of unknown nature as in hereditary spherocytosis or by various types of acquired injury In acquired hæmolytic anæmia of the auto antibody type a varying degree of spherocytosis and increase in osmotic fragility are almost invariably found in patients in active phases of the disease (see Fig 63 p 173) in animals in which hæmolytic anæmia is produced experimentally by the injection of anti erythrocyte immune sera marked degrees of spherocytosis are invariable if a sufficient dose of the hæmolytic serum has been

increase in erythrocyte fragility as the result of incubation *in vitro* A rapid rate of autohæmolysis is in fact commonly found in auto immune hæmolytic anæmia (Selwyn and Dacie 1954 and Fig 20 p 27) In Cases 11 and 12 (pp 201–205) autohæmolysis was extremely rapid despite the fact that there was no evidence that the antibodies had any direct hæmolytic potency The hypothesis that antibodies adsorbed to erythrocytes cause spherocytosis slowly and indirectly by affecting the metabolism of the cell surfaces rather than by direct damage also provides an explanation for the failure of antibodies to cause any rapid development of spherocytosis *in vitro*

Selwyn (1953) studied five patients in three of them the increase *in the erythrocyte osmotic fragility as the result of incubation for* 24 hours exceeded that of normal blood and in four out of the five patients glucose had less than its normal effect in diminishing hæmolysis In one seriously ill patient the presence of glucose had absolutely no effect in diminishing the rapid rate of autohæmolysis These studies therefore provide some evidence of an altered erythrocyte metabolism but whether this is due directly to damage caused by adsorbed antibody or to damage brought about indirectly as the result of the effects of intravascular agglutination as suggested by Castle Ham and Shen (1950) remains to be determined

Spherocytosis when once produced in acquired hæmolytic anæmia is probably irreversible and spherocytes almost certainly have a shortened life span Some probably undergo rapid lysis within the spleen others probably break up in the peripheral circulation perhaps because of their increased sensitivity to mechanical trauma

Clinically the presence of a moderate or marked degree of spherocytosis is usually associated with a serious rate of hæmolysis The extremely marked spherocytosis of the erythrocytes of Case 1° the patient whose serum contained an auto agglutinin active at 37 C and who died in a hyperacute hæmolytic crisis may be quoted as an instance of this association

It has already been mentioned that spherocytosis may be inconspicuous and osmotic fragility normal in patients whose erythrocytes nevertheless react strongly with antiglobulin serum (e g Case 9) Why this should be awaits elucidation

Erythrophagocytosis Phagocytosis seems to be one mechanism by which erythrocytes sensitized by certain antibodies (or damaged by other means) are disposed of As has already been mentioned on p 171 erythrophagocytosis has occasionally been observed in the peripheral blood of patients suffering from acquired hæmolytic anæmia In sections of patients spleens

and Richardson (1953) They showed that the corpuscles of guinea pigs injected with rabbit anti guinea pig erythrocyte sera became coated not only with rabbit serum protein but also with guinea pig serum protein In guinea pigs previously immunized against rabbit serum the guinea pig erythrocytes quickly became heavily coated with homologous protein and it is interesting to note that no hæmolytic anæmia then developed The auto sensitization observed by Muratore and co workers and by Samaille and Richardson seems therefore to be due to the reaction between anti erythrocyte antibody of the rabbit serum and guinea pig anti rabbit serum globulin taking place on the surface of the guinea pig s corpuscles Some doubt seems to be cast upon Muratore and co workers interpretation of their observations as the result of the work of Samaille and Richardson for the latter s experiments suggested that the auto sensitization was protective rather than harmful

One other protective mechanism deserves mention Cruz and Junqueira (1952) have shown that the resistance of reticulocytes to hæmolytic sera *in vitro* is from two to four times greater than that of non reticulated erythrocytes Cruz and Junqueira suggested that this helped to explain the extremely high reticulocyte counts seen in acute hæmolytic episodes

The observations that have been made on the experimental hæmolytic anæmias produced by the injection of anti erythrocyte sera into animals are of great significance in relation to the pathogenesis of human acquired hæmolytic anæmia and have conclusively proved that spherocytosis can result from antibody action However Dameshek and Schwartz s (1938) original view that spherocytosis was brought about directly as the result of the action of hæmolysin upon the surfaces of the erythrocytes has had to be modified As mentioned on p 300 Castle Ham and Shen (1950) concluded that spherocytosis developed when agglutinated erythrocytes were exposed to injurious products derived from degenerating tissues and that the tissue degeneration might be caused by ischæmia resulting from autohæmagglutination This hypothesis provided an explanation for the development of spherocytosis *in vivo* by antibodies which did not seem capable of producing this change *in vitro* it also accounted for the *pronounced hæmolysis in vivo caused by antibodies* which were only weakly or not at all hæmolytic *in vitro*

There is however another way in which spherocytosis might be produced It is conceivable that antibodies adsorbed by erythrocytes might so interfere with the metabolism at the surface of the corpuscles as to cause irreversible degeneration of the cell membranes This would be expected to result in time in spherocytosis and erythrocyte destruction *in vivo* and also in a rapid rate of autohæmolysis and perhaps an unusually great

organ there is often a considerable degree of hæmosiderosis and in many cases phagocytes containing ingested erythrocytes can be identified without much difficulty The bilirubin content of blood from the spleen or splenic vein may also be considerably higher than that of venous blood obtained from a peripheral vein (see p 207 Case 13)

The role of the spleen in experimental hæmolytic anæmia has received attention ever since experiments were started at the beginning of the century and the marked congestion with blood and the abundant evidence of erythrophagocytosis and siderosis within the spleen following the administration of a hæmolytic immune serum or chemical have been remarked upon by many workers (Levaditi 1902 Dudgeon Panton and Ross 1909 Banti 1913 Baumgartner 1947 Bessis and Freixa 1947 etc)

The effect of the previous removal of an animal s spleen on its subsequent sensitivity to an immune serum or hæmotoxic chemical has been the subject of a number of studies

Banti (1913) found that the hæmolytic effect of an anti erythrocyte serum and of toluylendiamine was less marked in dogs and in rabbits after their spleens had been removed These observations were confirmed by Pearce Krumbhaar and Frazier (1918) in dogs and also by Wasastjerna (1951) in guinea pigs Piovella and Formaggio (1950) found that dogs were slightly more resistant after splenectomy and Piovella (1953) observed that dogs whose spleens had been caused to contract by the administration of acaprine a quinine derivative were similarly less sensitive to anti erythrocyte serum On the other hand Tischendorf and Franke (1950–1) concluded that splenectomy had no significant influence on the course of experimental hæmolytic anæmia in rats

The studies outlined above provide on the whole reasonably good evidence for the amelioration by previous splenectomy of the hæmolytic effects of immune sera in some species Presumably the benefit is due to the removal of an organ in which auto hæmagglutination may cause local arrest of the circulation and which is in addition an important site of erythrophagocytosis However it is clear from the experimental reports that the effects of splenectomy are quantitative with a given dose of immune serum the resulting hæmoglobinæmia hæmoglobinuria anæmia spherocytosis and increase in osmotic fragility are likely to be slightly to moderately reduced but not abolished

In human cases of acquired hæmolytic anæmia as opposed to the experimental disease in animals there is the additional possibility that the spleen is an important site of auto antibody formation and that this may sometimes be the explanation of a sudden

removed at operation evidence of the phagocytosis of erythrocytes by macrophages is usually easy to find In experimental hæmolytic anæmia due to the injection of immune anti erythrocyte sera erythrophagocytosis is often conspicuous in internal organs particularly in the spleen and to some extent also in the peripheral blood (Levaditi 1902 Dudgeon Panton and Ross 1909 Baumgartner 1947 Bessis and Freixa 1947 Wasastjerna 1951 1953) It should be added that there seems no reason to believe that excessive phagocytosis of normal undamaged corpuscles is of any importance in the pathogenesis of idiopathic acquired hæmolytic anæmia

That certain types of auto antibody are capable of sensitizing normal human erythrocytes to phagocytosis *in vitro* has been shown by Bonnin and Schwartz (1954) working in the author s laboratory In experiments using both warm and cold antibodies from patients suffering from acquired hæmolytic anæmia they showed that only antibodies capable of causing the fixation of complement and hence hæmolysis caused the phagocytosis of erythrocytes by neutrophil polymorphonuclears and/or by monocytes

These laboratory studies do not tally exactly with the observations that have been made on erythrophagocytosis *in vivo* For instance erythrophagocytosis by monocytes has been observed in peripheral blood films made from patients whose auto antibodies have been found to be incapable of causing rapid hæmolysis *in vitro* e g Case 12 (Fig 64 p 176) whose antibody had the anti Rh specificity anti C and anti e No doubt though conditions in life are more favourable for vital phenomena such as phagocytosis *than are the highly artificial test tube conditions of the* laboratory Moreover it has been claimed that Rh antibodies do in fact slowly cause hæmolysis of normal corpuscles *in vitro* (Hill Haberman and Jones 1948 Ballowitz and Ballowitz 1954) but it remains to be seen whether the observed hæmolysis is due to the combined action of complement and antibody or due to the acceleration by the adsorbed antibodies of the autolysis which normally takes place when blood is incubated for 24 to 48 hours

Role of the Spleen

It is common knowledge that the spleen is probably always enlarged in human acquired hæmolytic anæmia and that splenectomy often has a favourable influence on the course of the disease (see Chapter 12) Sections of human spleens usually provide definite evidence of the occurrence of hæmolysis within the

hæmolytic anæmia or for that matter the reasons for success in some cases are far from clear

Mechanism of Erythrocyte Destruction *in vivo* in Acquired Hæmolytic Anæmia of the cold antibody type

As shown in Chapter 7 the concentration of cold antibodies in the sera of patients suffering from acquired hæmolytic anæmia may be very high indeed whilst the temperature at which the antibodies are active *in vitro* may extend almost if not quite to 37 C It seems reasonable to regard the temperature up to which the antibodies are active as of more importance to the patient than the titre of the antibodies at a low temperature

As described on p 184 cold antibodies are capable of causing both agglutination and lysis of normal corpuscles *in vitro* as well as sensitization to antiglobulin sera Normal corpuscles are however much less sensitive to lysis than to agglutination even if the pH for lysis is adjusted to the optimum (usually pH 6 5–7 0) However in some cases hæmolysis may be observed to take place *in vitro* at a relatively high temperature (30 C) and at the physiological pH of blood That at least part of the hæmolysis takes place in some cases in the blood stream is shown by the occasional episodes of hæmoglobinuria and the more constant presence of minor degrees of hæmoglobinæmia and of hæmosiderin in the urinary deposit (Crosby and Dameshek 1951) It has been claimed (Stats 1945) that it is the sensitivity of agglutinated corpuscles to mechanical trauma that is responsible for their breakdown in the blood stream when the temperature of the blood falls below 37 C That auto agglutination of the patients corpuscles may take place under natural conditions is shown by the frequent occurrence of Raynaud s phenomena in patients whose sera contain high titre cold antibodies (see p 175) It must be borne in mind however that antibody complement lysis can be demonstrated to take place *in vitro* up to about the same temperature at which normal corpuscles are agglutinated

Ham Gardner Wagley and Shen (1948) concluded that mechanical trauma caused lysis only when the corpuscles were already sensitized by incomplete antibodies They studied two patients whose sera contained cold agglutinins at the same concentrations (titre 5 000) One patient who was suffering from acquired hæmolytic anæmia was anæmic her erythrocytes gave a positive direct antiglobulin test the other patient convalescing from virus pneumonia was not anæmic and her erythrocytes were not agglutinated by antiglobulin serum

cessation of hæmolysis following splenectomy Unfortunately there is little reliable information on this point

Evans and Duane (1949) studied two patients and found that a diminution in the rate of hæmolysis after splenectomy was associated in each case with a striking reduction in the agglutinability of the patients erythrocytes by antiglobulin serum

Several of the author s patients have also been studied in some detail before and after splenectomy In the patient described as Case 9 the concentration of antibodies adsorbed to her corpuscles remained virtually unchanged as judged by the strength of the direct antiglobulin reaction over a period of six and a half years after splenectomy She nevertheless experienced a sustained clinical remission and there seemed little doubt that the rate of hæmolysis had been substantially reduced by removal of her spleen (Fig 68 p 197) The indirect antiglobulin reaction however became negative suggesting that there had in fact been some diminution in the rate of antibody production

The patient described as Case 18 has also been studied at frequent intervals since splenectomy Considerable clinical benefit resulted and there seemed no doubt that the rate of hæmolysis was diminished (Fig 22 p 35) However the intensity of the sensitization of her erythrocytes and the antibody titres in her serum remained apparently unaltered (Fig 70 p 207) In this patient at least it seemed that the spleen was acting more as a hæmolytic organ than as a site of formation of auto antibodies

It is admittedly difficult to understand how and why highly sensitized corpuscles (as judged by the antiglobulin reaction) survive so well in patients in clinical remission as for example in Case 9 after splenectomy Whether the removal of a phagocytic organ and a moderate reduction in the amounts of antibody formed provide a complete explanation for these patients remissions is uncertain

If splenectomy is sometimes successful in bringing about clinical cure of patients in whom antibody formation persists why does this not always happen? It appears likely to the author that in most cases where splenectomy fails overwhelming amounts of antibody potent in causing erythrocyte destruction are being produced with the result that cell survival *in vivo* is grossly shortened Under these circumstances a moderate improvement in the survival of the patient s corpuscles is of little practical value to him However it has to be admitted that the reasons for failure of splenectomy in idiopathic acquired

titres were significantly less—neither patient suffered from hæmolytic anæmia

TABLE 25 *A comparison between the agglutinin and hæmolysin titres of the sera of five patients who had probably suffered from virus pneumonia Patients Pa Ma and Sl developed acute hæmolytic anæmia patients Ra and Ba did not become anæmic*

Patient	Agglutination of normal erythrocytes (Titre at 17° C)	Hæmolysis of P.N.H. erythrocytes (Titre at 1 C)
Ra	256	64
Ba	512	32
Pa	51_	256
Ma	512	256
Sl	512	256

A patient suffering from hæmolytic anæmia following virus pneumonia (Case 16) provided an opportunity for studying the reactions of his antibody *in vitro* in relation to the progress of his clinical recovery (Fig _3) Four samples of serum were compared for their ability to hæmolyse P N H erythrocytes at 37° C—a measure of antibody action at the upper limit of its thermal range—and for their ability to agglutinate normal erythrocytes at 2 C Clinical recovery was associated with loss of activity at 3_° C but only a small change in the agglutinin titre at 2 C

Significance of Complement Changes

A factor of at least theoretical importance in relation to hæmolysis by the antibodies of acquired hæmolytic anæmia is the level of serum complement Experimentally it has been shown that the rapidity of hæmolysis *in vivo* of corpuscles sensitized by hæmolysins is dependent upon the amount of complement present (Christian Stewart Yuile Ervin and Young 1951) A reduction in the level of serum complement has been reported in certain cases of acquired hæmolytic anæmia (see p 195) The results of complement titration carried out on the sera of some of the patients considered in Chapter 7 showed that four of them had subnormal levels However there did not seem to be any correlation between the results of complement titration and the

Chilling the patients arms for 20 minutes resulted in hæmoglobinæmia in the first patient but not in the second

Stats s (1945) observations were also against the hypothesis that the intensity of hæmolysis *in vivo* can be correlated with the cold agglutinin titre (at 0 to 2 C) For example one patient with a hæmagglutinin titre of 10 000 had experienced an attack of acute hæmolytic anæmia three others however with hæmagglutinin titres of between 5 120 and 12 800 had neither hæmolytic anæmia nor hæmoglobinuria

Of the patients in the author s series those suffering from hæmolytic anæmia following virus pneumonia (e g Cases 15 and 16) developed acute anæmia with antibody concentrations considerably lower than those of some of the patients with the idiopathic disease in whom the rate of erythrocyte destruction appeared to be considerably less It seems likely that qualitative differences in the antibodies such as the ability to agglutinate or sensitize the patients erythrocytes at relatively high temperatures and to fix complement and bring about hæmolysis at the physiological pH of blood are important Of the patients with very high titre cold antibodies who suffered from Raynaud s phenomena in cold weather it was the patient whose antibody caused marked lysis of normal corpuscles in *unacidified* patient s serum *in vitro* (see p 251) who suffered from frequent attacks of hæmoglobinuria On the other hand attacks of clinical hæmoglobinuria were infrequent or absent in the patients whose serum had to be acidified in order to demonstrate its hæmolytic property *in vitro* (e g Case 14)

It is interesting to note that in some patients whose sera contain cold antibodies hæmolysis does not seem to be affected to any great extent by the seasonal variations of temperatures in London (e g Case 14) In these patients it is presumably the fact that antibody activity extends up to 37 C (at least as judged by sensitization to antiglobulin serum) that is the cause of the continuation of hæmolysis in warm weather and when the patient is kept warm in bed

Other evidence which suggests a correlation between clinical hæmolysis and the hæmolytic power of the patients sera *in vitro* is summarized in Table 25 Here are compared the agglutinin and hæmolysin concentrations in five sera obtained from patients who probably had suffered from virus pneumonia titrated with normal and P N H erythrocytes respectively The agglutinin titres were about the same in each instance The hæmolysin titres using the P N H corpuscles varied in patients Pa Ma and Sl they were close to the agglutinin titres—they all suffered from hæmolytic anæmia in patients Ra and Ba the hæmolysin

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probable rates of hæmolysis *in vivo* For instance the serum complement titre of Case 13 was persistently low following splenectomy despite the fact that the rate of hæmolysis had been substantially reduced following the operation It seems likely that alterations in serum complement activity are often merely manifestations of an abnormal development of plasma protein It is interesting to note that the sera of all the patients whose complement activity was abnormally low contained cold auto antibodies predominantly and not warm ones

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and Heck (1948) reported three failures five patients improved and ten excellent results Robson (1949) reported that five out of seven chronic cases and two acute cases were benefited by splenectomy and Dreyfus Dausset and Vidal (1951) had good results in two out of five cases

Unfortunately it does not yet seem possible to predict which patients will respond favourably to removal of their spleens and which will not either on clinical hæmatological or serological grounds Of sixteen patients the author has investigated who have undergone splenectomy nine have enjoyed sustained remissions lasting between six months and seven years of the remaining seven five died of their disease despite splenectomy and two went into remissions as the result of A C T H therapy given after splenectomy had failed The auto antibodies were of the warm variety in six of the patients favourably affected (e g Case 9) and of the cold type in three patients (e g Case 13) The extent of the increase in erythrocyte osmotic fragility did not seem to have any prognostic significance for the fragilities of the patients favourably affected varied from within the normal range to a marked increase The role of the spleen in acquired hæmolytic anæmia and the possible ways in which splenectomy may bring about a reduction in the rate of hæmolysis have already been discussed (p 304).

Blood Transfusion

Transfusion as a means of therapy for acquired hæmolytic anæmia was undoubtedly popularized by the publication of Lederer (1925) who described three patients suffering from acute hæmolytic anæmia in whom recovery seemed to be initiated by transfusion In a later report Lederer (1930) reviewed twelve cases and considered that eleven of them had responded to transfusion It appears extremely doubtful in retrospect whether transfusion played a decisive part in the recovery of these patients except in as much as it helped to tide them over their anæmic crises The recoveries appear as likely to have been spontaneous as due directly to the transfusions Whether or not normal plasma or serum contains any or sufficient anti lytic substances to have a specific inhibitory effect on hæmolysis remains doubtful (see p 245) Certainly transfusion is seldom followed by dramatic or sustained benefit in the majority of cases of acquired hæmolytic anæmia seen to day Dameshek and Rosenthal (1951) reviewing their own experience stated that in only eight out of 70 cases of acquired hæmolytic anæmia of mixed pathogenesis were trans

CHAPTER 12

ACQUIRED HÆMOLYTIC ANÆMIA (AUTO ANTIBODY TYPE)

VI TREATMENT

The treatment of patients suffering from acquired hæmolytic anæmia of the auto antibody type is still largely empirical Until quite recently there were only two well established but by no means *always successful lines of treatment namely splenectomy* and blood transfusion Now with the advent of adrenocorticotropic hormone and cortisone a third and sometimes very potent form of therapy is available These three main methods of treatment will be dealt with in historical order beginning with splenectomy *and ending with A C T H and cortisone* Finally some other types of therapy designed to depress antibody formation will be briefly mentioned

Splenectomy

The beneficial effect of splenectomy in acquired hæmolytic anæmia seems to have been reported for the first time by Micheli in 1911 Other favourable accounts soon followed (e g Antonelli 1913 Nobel and Steinebach 1914) and by 1940 Dameshek and Schwartz were able to collect together reports of 23 patients suffering from the acute form of the disease (including four patients of their own) twenty of whom had responded favourably to splenectomy Later Dameshek (1943) reported good results in ten out of 18 personally studied patients

Many subacute and chronic cases also benefit greatly from splenectomy However not infrequently the operation fails either hæmolysis continues apparently unabated or after an initial favourable response lasting days weeks or even months the patient relapses again and becomes as seriously ill as he was before splenectomy According to Welch and Dameshek (1950) who reviewed 34 cases of idiopathic acquired hæmolytic anæmia *splenectomy is followed by a complete remission in approximately* 50% of patients Rather similar results based on smaller series of patients have been published by others For example Stickney

and Heck (1948) reported three failures five patients improved and ten excellent results Robson (1949) reported that five out of seven chronic cases and two acute cases were benefited by splenectomy and Dreyfus Dausset and Vidal (1951) had good results in two out of five cases

Unfortunately it does not yet seem possible to predict which patients will respond favourably to removal of their spleens and which will not either on clinical hæmatological or serological grounds Of sixteen patients the author has investigated who have undergone splenectomy nine have enjoyed sustained remissions lasting between six months and seven years of the remaining seven five died of their disease despite splenectomy and two went into remissions as the result of A C T H therapy given after splenectomy had failed The auto antibodies were of the warm variety in six of the patients favourably affected (e g Case 9) and of the cold type in three patients (e g Case 13) The extent of the increase in erythrocyte osmotic fragility did not seem to have any prognostic significance for the fragilities of the patients favourably affected varied from within the normal range to a marked increase The role of the spleen in acquired hæmolytic anæmia and the possible ways in which splenectomy may bring about a reduction in the rate of hæmolysis have already been discussed (p 304).

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fused plasma rather than to hæmolysis This type of plasma reaction described by Dameshek and Neber (1950) and Dameshek and Rosenthal (1951) can be avoided by the transfusion of erythrocytes washed in saline According to Crosby and Stefanini (1952) the plasma reaction is due to an unidentified heat labile factor At the time of the reaction the leucocyte and platelet counts and the fibrinogen concentration are lowered Crosby and Stefanini suggested that the symptoms of the reaction might be caused by vascular obstruction due to small emboli

Cross matching of Blood for Transfusion This is a source of difficulty and anxiety as the serum of most patients will be found to contain non specific abnormal antibodies if their disease is active It is likely therefore that apart from exceptional cases normal blood samples will appear to be more or less incompatible if compatibility tests are carried out by sensitive methods such as the indirect antiglobulin test (see p 486) The best that can be done is to select from specimens of blood of homologous ABO group and Rh type those samples which appear to be least incompatible when the cross matching test is carried out at 37 C The patient s blood should be genotyped before he receives his first transfusion and the specificity of his auto antibodies determined if possible This knowledge may enable the serologist to select compatible blood and it will also indicate what types of iso antibodies a patient receiving many transfusions is likely to develop

If blood known to be more or less incompatible has to be administered to a patient it should be given slowly The rate should certainly not exceed 100 ml of packed corpuscles per hour If the patient is regularly intolerant of transfusions it is worthwhile seeing whether a transfusion of saline washed corpuscles is better tolerated

Treatment with A C T H and Cortisone

The results so far obtained by treatment with A C T H and cortisone have been interesting and moderately encouraging Many patients with acquired hæmolytic anæmia of the auto antibody type derive benefit but it must be admitted that the results are rather unpredictable and that it is far from clear how the beneficial effect of treatment is brought about

Literature on A C T H and Cortisone Therapy

The first published reports date from 1950 Dameshek (1950) reported startling ' improvement as the result of treating with

fusions followed by complete remissions and in only two of the remitting cases was the disorder of the auto antibody type

In the great majority of patients suffering from acquired hæmolytic anæmia transfusion therefore cannot be expected to be more than palliative and its value is seriously limited by the fact that the survival of the normal blood in the patient is likely to be no better than that of his own erythrocytes (see however the account of Case 12 whose antibody had a definite specificity) The almost invariable result is that except in mild cases transfusion can be of only transient benefit and the improvement if any in the patient s general condition is usually only a matter of days at the most A very seriously ill patient may actually appear worse after transfusion for the benefit due to a rise in hæmoglobin will be transient and the transfusion by providing him with more cells to destroy will inevitably result in an increased rate of bilirubin formation with consequent increase in jaundice In other patients in whom hæmolysis is taking place in the blood stream transfusion may merely result in an increase in hæmoglobinuria as in Case 11

Nevertheless despite the limited value of transfusion in serious cases it is impossible to let the patient die of anæmia untransfused In patients who are severely anæmic A C T H or cortisone therapy (see p 323) should certainly be started at the same time as transfusion In less seriously ill patients transfusion may be useful as a preparation for splenectomy and in obscure cases a determination of the survival of the normal blood may help in diagnosis Exsanguination transfusion has been attempted on several occasions but the benefit is usually only transient (see *Milliez et al* 1951)

It has been commonly held that reactions occur frequently when transfusions are given to patients with acquired hæmolytic anæmia This is probably true although in most patients slight fever and an intensification of the patients jaundice are the only accompaniments There are several reasons for the frequency of reactions not only may non specific or specific auto antibodies be present in the patient s plasma but he may have developed immune iso antibodies as a consequence of past transfusions—the relative frequency of anti E has already been mentioned (p 195) Occasionally patients are met with who appear to be regularly intolerant of transfusions and who develop pyrexia and rigors and may complain of backache after relatively small amounts of apparently compatible blood have been transfused In certain patients this syndrome appears to be due to sensitivity to trans

sort of favourable response to treatment in only three was the result really good: in these three cases the improvement was maintained for more than six months after stopping treatment. In some of the patients who responded partially the antiglobulin reaction became weaker; in others who responded the test still remained positive. The later history of some of these patients has recently been recorded (M.R.C. Hæmatology Panel 1953). Of the three patients who responded well one relapsed and died, the other two remained well. Of the five patients who responded partially one recovered after splenectomy, but three died; one patient was not traced.

The results of Meyers, Miller, Linman and Bethell (1952) were more encouraging: complete remissions developed in six out of seven patients apparently suffering from the idiopathic type of the disease. Daily doses of 100 to 160 mg. of A.C.T.H. or up to 300 mg. of cortisone were given. One patient remained in complete remission for 15 months after discontinuing the hormone therapy, two relapsed partially and four patients relapsed completely and needed further treatment. Meyers and co-workers concluded that the best results seemed to be obtained in patients who responded to moderate amounts of the hormones after relatively short periods of treatment.

The results of treatment of four more idiopathic cases were reported by Davis, Kennedy, Baikie and Brown (1952). Two of the patients responded favourably; two derived little or no benefit.

More recently Dameshek (1952) in commenting on the relatively poor results of the British M.R.C. trial attributed this to a too small scale of dosage. With daily doses up to 300 mg. of A.C.T.H. or cortisone Dameshek found that hæmolysis could nearly always be controlled; he stated that fourteen out of 22 patients experienced complete hæmatological and clinical remissions, although the direct antiglobulin tests more often than not remained positive. Rosenthal, Spaet, Goldenberg and Dameshek (1952) used compound F. When given intramuscularly compound F appeared to be less effective than A.C.T.H. or cortisone in four patients; when given orally compound F produced a good remission in one patient, but in another it seemed to be less effective than cortisone.

Rose and Nabarro (1953) studied three children severely ill with acute hæmolytic anæmia. Repeated transfusions did not affect the rate of hæmolysis; all three, however, responded to relatively large doses of A.C.T.H. or cortisone. One child recovered

A C T H two patients who were suffering from acquired hæmolytic anæmia associated with generalized lymphosarcoma the serum bilirubin concentration and the antibody content in their sera diminished their blood counts rose and the lymphosarcoma regressed Dameshek also reported that two other patients with the idiopathic disease who had not responded to splenectomy improved on A C T H therapy Gardner (1950) described improvement in three patients in one a girl aged five years the erythrocyte osmotic and mechanical fragilities returned to normal and there was a fall in the Coombs titre

Details of five patients were given by Dameshek Rosenthal and Schwartz (1951) In each case the direct antiglobulin test was positive and all had circulating antibodies Three of the patients were suffering from symptomatic hæmolytic anæmia associated with lymphosarcoma or lymphatic leukæmia whilst in two patients the disease was of the idiopathic type All received intensive A C T H therapy dosage varied from 30 mg to 80 mg given intramuscularly at 6 or 8 hour intervals Four of the five patients underwent almost complete remissions and their antibody titres were markedly diminished Two of the patients relapsed following cessation of therapy but re administration of A C T H resulted in further remissions In a footnote the authors referred to three other patients suffering from the idiopathic type of the disease all of whom responded dramatically to treatment with A C T H

Gardner McElfresh Harris and Diamond (1951) reported detailed studies in three patients suffering from the idiopathic disease two were children one was an adult The direct Coombs titre and the *erythrocyte mechanical fragility declined markedly* in each case and in two patients the erythrocyte osmotic fragility became normal Daily treatment with 100 mg of A C T H resulted in the disappearance from the adult patient s serum of an agglutinin and hæmolysin active against normal corpuscles at *p*H 6 4 At the same time there was a diminution in the concentration of serum γ globulins

Wintrobe Cartwright Palmer Kuhns and Samuels (1951) reported the results of treating three patients with A C T H the maximum dosage being 100 mg to 200 mg daily In one idiopathic case a striking remission lasting more than nine months followed the daily administration of 200 mg of A C T H the direct Coombs titre however increased The other patients suffering from *chronic lymphatic* leukæmia and from disseminated lupus erythematosus respectively responded moderately well in the latter patient the antiglobulin test became negative

The results of the trials sponsored in Britain by the Medical Research Council were only moderately encouraging (M R C Hæmatology Panel 1952) The usual minimum course of treatment was 1 g of A C T H or 1 5 g of cortisone given over a period of ten days Although eight out of eleven patients showed some

proved ineffective and de Gruchy (1954) referred to four patients who responded to A C T H but not to cortisone

Mode of Action of A C T H and Cortisone The exact way in which A C T H and/or cortisone bring about benefit in acquired hæmolytic anæmia is still not clear In some of the patients who respond there seems to be a reduction in the concentration of abnormal antibodies in the serum or in the strength of the direct antiglobulin test (e g Saint and Gardner 1952) exceptionally auto antibodies cease to be demonstrable (Dameshek 1952 Mallarmé 1953) In other patients the direct antiglobulin test remains strongly positive despite clinical improvement (Clearkin 1952) This was also true of two of the patients studied by the author (Cases 10 and 14) in neither of whom could a significant change be demonstrated in the reactions of their corpuscles in the direct antiglobulin test or in the concentrations of antibodies in their sera compared with serial observations made before the hormones were given Collateral evidence obtained in man which suggests that there may nevertheless be depression of antibody formation in at least some cases is provided by the observation that the concentration of serum γ globulin may be reduced following the administration of A C T H (Vaughan Bayles and Favour 1950 Gardner *et al* 1951 Saint and Gardner 1952 and Hansen 1953)

Other possible methods of the action of A C T H and/or cortisone include an effect on the marrow resulting in accelerated erythropoiesis (Hudson Herdan and Yoffey 1951) interference with the reaction between the erythrocytes and antibodies as the result of an effect of cortisone on cell permeability (Thorn *et al* 1950) and inhibition of erythrophagocytosis

Despite the inconstancy of the effects of treatment on the antibodies in human cases of acquired hæmolytic anæmia there is some experimental evidence which indicates that the prolonged administration of adrenocorticotrophic hormones to animals leads to a reduction in the concentration of circulating antibodies

For instance Bjorneboe Fischel and Stoerk (1951) demonstrated in rabbits a reduction in the concentration of antipneumococcal antibodies and de Vries (1950) also in rabbits a reduction of the concentration of antibodies against egg albumen as a result of the administration of A C T H Similarly Germuth Oyama and Ottinger (1951) showed that compound L (cortisone) given to rabbits sensitized with egg albumen markedly inhibited the development of anaphylactic hypersensitivity of the Arthus type The effect seemed to be due to a reduction in the rate of formation of antibody rather than to acceleration of antibody destruction for no effect on the rate of disappearance

after five weeks of A C T H therapy the other two relapsed when the drug was withdrawn but remitted when A C T H was re administered One child recovered after three courses of A C T H and cortisone hæmolysis in the other persisted longer but was still being controlled by a daily dose of 75 mg of cortisone 30 weeks after the start of his illness The sera of all three children contained abnormal auto antibodies Aber Chandler and Hartfall (1954) reported that the last patient was still being maintained in good health on 75 mg of cortisone a day 60 weeks after commencement of treatment

The British M R C Hæmatology Panel have recently published a second (1953) report Ten further patients have been treated in seven the hæmolytic anæmia was idiopathic in three it was of the secondary type In seven patients the antiglobulin test was positive Five patients underwent complete remissions and three partial remissions Two patients failed to respond (both had negative direct antiglobulin tests) their resistance could not be ascribed to under dosage Throughout the series the daily dosage of the drugs ranged from 80 to 200 mg of A C T H and 100 to 300 mg of cortisone

Other favourable reports based on the study of single cases include those of Davidson Duthie Girdwood and Sinclair (1951) Etess Bassen Litwins and Sussman (1951) Unger (1951) Meyer (1951) Crary and Beck (1952) Saint and Gardner (1952) Gunz and Aiken (195) and Aitchison (1953)

Best Limarzi and Poncher (1951) reported improvement in two cases Clearkin (1952) in one out of two patients Hansen (1952) good results in three out of four patients and Sacks Workman and Jahn (1952) remissions in two patients and partial remissions in six others

Three patients of the author s series have been treated intensively with A C T H and/or cortisone All three responded partially to the drug one patient (Case 21) suffering from a secondary hæmolytic anæmia of the cold antibody type appeared to go into an almost complete remission for a time Of the idiopathic cases who partially remitted one patient (Case 10) had antibodies of the warm type the other (Case 14) high titre cold antibodies

Most workers have assumed that A C T H and cortisone have the same effect in comparable doses Although this is probably true in the great majority of instances the possibility of cortisone being effective when A C T H fails and *vice versa* should be borne in mind Aitchison (1953) has published an account of a patient who reacted favourably to cortisone after A C T H had

however some features in common including the inconstant effect on antibody formation and the probability that any favourable effect they have on hæmolysis is brought about by several mechanisms

Practical Implications for Treatment

The outlook in acquired hæmolytic anæmia has undoubtedly been altered by the advent of A C T H and cortisone and there is now little doubt that the hormones should be the first choice of treatment for any patient who is seriously anæmic The patient must first of all be under the care of a physician who has had experience of the drugs general metabolic effects for they may have to be given for long periods of time in large doses e g in adults up to 300 mg per day of cortisone orally or up to 200 mg of A C T H or the equivalent as A C T H gel in divided intra muscular doses If and when the patient responds the dosage should be cut down to the minimum which keeps the patient in reasonable remission so as to economize the drugs and to avoid unpleasant side effects The aim should be to maintain a hæmo globin concentration of at least 11 g per 100 ml There seems no point in giving very large doses in an attempt to obtain a normal hæmoglobin concentration for the hormones are probably in no sense a cure for the patient s disease The best that can be hoped for is to control the severity of the hæmolysis until spon taneous recovery takes place This may necessitate continuing therapy for many months or possibly for years (Aber Chandler and Hartfall 1953)

Splenectomy should be seriously considered in any patient to whom A C T H or cortisone has to be given in large doses for long periods in order to obtain a favourable effect As already men tioned the consequences of the operation are unpredictable and in only about 50% of patients may good results be anticipated Nevertheless it seems reasonable to recommend splenectomy in all patients suffering from acquired hæmolytic anæmia of the idiopathic type unless they be very young or elderly if their anæmia has persisted at a serious level for months and if this shows no signs of abating or allowing a reduction in the dosage of A C T H or cortisone A favourable response to A C T H or cortisone cannot unfortunately be taken as an indication that splenectomy is likely to be successful Sufficient reports to the contrary are now available (e g Rose and Nabarro 1953) In severely ill patients who do not respond at all to the hormones splenectomy should be carried out if the patients can be made

of antibody could be demonstrated in passively sensitized rabbits The latter observation certainly fits in with the clinical observation that A C T H or cortisone given to the human subject in the treatment of acquired hæmolytic anæmia has *no immediate effect on the concentration* of circulating antibodies (Dameshek Rosenthal and Schwartz 1951) Clinical experience also agrees with experimental work in indicating that there is no release of antibody into the circulation in association with the lymphopenia which develops as an immediate effect of A C T H administration (Eisen *et al* 1947 Fischel Le May and Kabat 1949 de Vries 1950)

There does not seem however to be any evidence that the hormones can suppress the formation of anti erythrocyte antibodies in experimental animals injected with the erythrocytes of other species In rabbits for instance cortisone and A C T H appeared to have no influence on the formation of antibodies against guinea pig or dog erythrocytes (Clearkin 1952 Ecklebe and Sander 1952)

The possibility that cortisone or A C T H might interfere with or inhibit the reaction between antigen and antibody was tested experimentally by Clearkin (1952) who found that the administration of cortisone to guinea pigs made no difference to the rate of hæmolysis caused by the injection into the animals of an anti erythrocyte serum Ecklebe and Sander (1952) on the other hand found that in dogs given anti dog erythrocyte serum the degree of auto agglutination and the amount of antibody which might be eluted off the dogs erythrocytes were reduced It should be added that neither Fyquem (1951) Tischendorf Ecklebe and Thofern (1951 52) nor Feldman and Rachmilewitz (1954) were able to demonstrate in cats and dogs and rats given anti erythrocyte sera that the course of the experimental hæmolytic anæmia was in any way benefited by the concurrent administration of A C T H or cortisone

The evidence cited above on the effects of A C T H and cortisone on the development of hæmolytic anæmia is thus inconclusive It seems likely that in some patients at least the prolonged administration of A C T H or cortisone leads to an actual diminution in the amount of circulating antibody formed but whether this is the whole explanation for the action of the hormones is a matter for conjecture Probably it is not for remissions seem to occur without any demonstrable alteration in antibody concentrations The fact that the patients may nevertheless respond favourably to treatment suggests that the hormones protect the corpuscles from the harmful effects of the antibodies in other ways That A C T H and cortisone may act in cases of hæmolytic anæmia quite independently of their possible action on antibody formation is illustrated by the report of Feldman and Rachmilewitz (1952) who found that the hormones protected rats from the hæmolytic effects of acetylphenylhydrazine

The results of A C T H and cortisone therapy in man are a little less unpredictable than are the results of splenectomy They have

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fit enough for the operation when it seems clear that transfusion therapy is of limited value and if the patient s general condition is deteriorating There seems to be some justification for carrying out splenectomy when hormone therapy fails as it is probably wrong to suppose that splenectomy and A C T H and cortisone act in exactly the same way and through the same mechanisms The operation has in fact been followed by a satisfactory response in a few patients known to have failed to respond to the hormones (M R C Hæmatology Panel 1953)

Other Methods of Treatment

Nitrogen Mustard Dameshek (1951) reported the effects of intravenous nitrogen mustard given to four patients in an attempt to reduce by damaging their lympho reticular tissue the amount of antibody formed In one patient the treatment was followed by a fall in antibody titres and although thrombo cytopenia and leucopenia caused anxiety the patient went on to a complete recovery and remained well for at least two years subsequently The other three patients were not benefited Meyers and co workers (1952) treated one patient in a similar way but the only result was myeloid depression Other cytotoxic agents which have been employed include urethane and radio active gold (Dameshek 1951) Usually no benefit has resulted

Thorotrast and X radiation Evans and Duane (1947) described the result of the administration of thorotrast to one patient and the effects of X radiation directed to the mediastinum and abdomen of two patients The patient who received thorotrast had relapsed following splenectomy She appeared to experience a partial remission with a transitory arrest in the progress of her anæmia following the administration of the thorotrast a longer remission followed X radiation In the second patient X radiation was without effect on the hæmolytic process

The above mentioned experiences with cytotoxic chemicals radio active materials and X radiation do not suggest that these dangerous weapons are likely to be much used in the treatment of acquired hæmolytic anæmia Their value is unproven and they are certainly much less effective and far more dangerous than A C T H and cortisone

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Clinically hæmolytic anæmia may present in several ways in association with lymphadenoma Usually a progressive anæmia develops in an established case of lymphadenoma the patient becomes slightly jaundiced and examination of his blood reveals the signs of a chronic hæmolytic anæmia Exacerbations in the anæmia commonly parallel periods of increased activity of the disease as shown by pyrexia and by swelling of the lymph nodes and spleen (Fig 82) Less commonly hæmolytic anæmia dominates the picture and only late in the course of the disease at splenectomy or perhaps at autopsy is lymph node enlargement and splenomegaly due to lymphadenoma discovered Holler and Paschkis s patient two of Davidson s cases and Singer s (1936) patient were of the latter type Occasionally anæmia of hæmolytic type together with periodic fever are the presenting symptoms as in the hitherto unpublished case described below

Blood Picture and Serology As a rule no very distinctive or diagnostic appearances are to be seen in peripheral blood films Some spherocytosis is usual but this is not invariable Anisocytosis poikilocytosis and polychromasia are usually moderate Normoblasts may often be found in small numbers The direct antiglobulin test is only occasionally positive (see p 334

Case Report Hæmolytic Anæmia Associated with Lymphadenoma

Case 19 The patient (B R) was a man aged 51 years complaining of fever pain in his back and loss of appetite and weight for four months On admission into hospital on June 16th 1949 he was found to be pale thin and ill looking His spleen was palpable 2 cm below the left costal margin and there were two doubtfully enlarged axillary lymph nodes No other abnormal physical signs were found His urine was normal

He remained under observation in hospital with only a short break until his death on October 16th 1949 During the whole time he had an irregular and somewhat fluctuating fever ranging from 99 F to 104 F which was unaffected by the administration of antibiotics Repeated blood cultures and tests for agglutinins against organisms of the *Brucella* and the *Salmonella* groups were negative Finally on October th 1949 biopsy of an axillary lymph node revealed the presence of lymphadenoma

The patient s condition deteriorated progressively He became slightly jaundiced and complained of considerable generalized pruritus and his anæmia became more profound X ray therapy directed to his spleen and mediastinum was commenced on September 15th No real benefit resulted although the spleen became slightly smaller

Laboratory Investigations At his first admission the hæmoglobin concentration was 10 9 g per 100 ml and the total leucocyte count

CHAPTER 13

HÆMOLYTIC ANÆMIA IN ASSOCIATION WITH LYMPHADENOMA LEUKÆMIA AND RETICULO SARCOMA AND CARCINOMATOSIS

An increased rate of erythrocyte destruction quite commonly occurs in diseases not primarily affecting erythropoiesis or the erythrocytes The intensity of the hæmolysis varies from a silent degree only detectable by careful studies of erythrocyte survival to hæmolysis of such intensity that it dominates the clinical picture In the literature the terms *secondary* (Watson 1939) or *symptomatic* (Singer and Dameshek 1941) have been used to describe the hæmolytic anæmia from which these patients suffer Most of the recorded examples of overt hæmolytic anæmia of this type have been observed in association with lymphadenoma reticulosarcoma leukæmia (particularly chronic lymphatic leukæmia) myelosclerosis and carcinomatosis The literature has been reviewed recently by Paraf and Dausset (1952) The above mentioned types of secondary hæmolytic anæmia will be discussed briefly in this chapter leaving other secondary types such as the hæmolytic anæmias associated with uræmia liver disease and disseminated lupus erythematosus etc to be dealt with in Chapter 14

HÆMOLYTIC ANÆMIA IN LYMPHADENOMA

The association of hæmolytic anæmia and lymphadenoma was first reported apparently by Holler and Paschkis (1927) who found that the spleen removed from a patient suffering from hæmolytic anæmia of unknown origin was infiltrated with lymph adenomatous tissue Davidson (1932) described three further examples and another patient was reported by Singer (1936) who referred to eleven other case reports collected from the literature Watson (1939) mentioned three examples and Singer and Dameshek (1941) described an additional case Other cases have been reported more recently by Davis (1944) Gruelund (1947) Trinick (1949) Brown and Meynell (1949) Brown (1950) Sulzer (1952) Willcox (1952) Foster and Hutt (1953) and Hennemann (1953)





FIG 83 Ph tom cr graph of a section of the pleen of Case 19 The nodules of lyn pl ad noma are urrounded by a cuff of iron containing pigment 1) reaction × 17 and × 50

15 000 cells per c mm, with 81% neutrophils His blood group was A Rh positive

When readmitted on August 24th 1949, he was transfused with two pints of compatible normal group A blood On the following day his hæmoglobin was 9 0 g per 100 ml nine days later it had fallen to 5 4 g a loss of approximately 0 5 g per day From this point his anæmia became progressively more severe and he was transfused six more times before his death on October 16th 1949

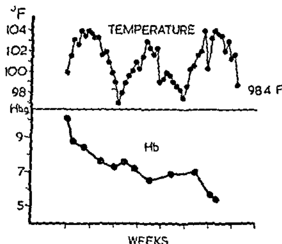


FIG 87 The relationship between anæmia and pyrexia in a patient suffering from lymphadenoma and hæmolytic anæmia The maximum daily temperature of the patient is recorded

On September 2nd 1949 he was transfused with the packed erythrocytes from four pints of group O Rh positive blood The survival of this blood was followed by the Ashby method using an anti A serum Approximately 50% of the transfused cells had been eliminated within eight days of the transfusion practically all were eliminated in 24 days (Fig 86 p 341)

His reticulocyte count varied between 5% and 14% and the total leucocyte count fluctuated between 9 000 and 26 000 cells per c mm The M C V was at the upper margin of normal and stained films showed considerable anisocytosis poikilocytosis polychromasia and punctate basophilia Spherocytes were not seen Small numbers of myelocytes were constantly present and also occasional normoblasts There were 18,000 platelets per c mm The osmotic fragility and the rate of autohæmolysis in vitro were normal

The direct antiglobulin test was negative the cold agglutinin titre was 4 No abnormal warm antibodies could be demonstrated in his serum by the indirect antiglobulin method The highest recorded bilirubin level was 2 0 mg per 100 ml The plasma protein concentrations were normal

Postmortem Examination The lymph nodes were enlarged throughout

the body the abdominal and thoracic nodes being larger than those at the periphery None was greater than [illegible] cm in diameter they were firm and discrete and homogeneous on section The spleen was large and weighed 900 g on section it showed numerous small whitish nodules suggestive of lymphadenoma its brownish colour suggested marked hæmosiderosis The bone marrow was extensively infiltrated by lymphadenomatous tissue throughout the skeleton Red marrow extended throughout the shaft of the femur

Histological sections of the enlarged lymph nodes and the deposits in the spleen showed the characteristic changes of lymphadenoma A most remarkable appearance was found in the spleen where each lymphadenomatous nodule was surrounded by a ring of intracellular and extracellular hæmosiderin at the point of contact between the lymphadenomatous tissue and the spleen pulp (Fig 83) The liver was free from infiltration The bone marrow was however extensively infiltrated

Summary A fatal case of lymphadenoma with massive involvement of the spleen and bone marrow and to a lesser extent of the lymph nodes Clinically the patient presented first with pyrexia of unknown origin and later as a severe anæmia of hæmolytic type

Hæmolytic Anæmia in Association with other Reticuloses

Anæmia probably in part hæmolytic in origin is a feature of histiocytic medullary reticulosis and similar obscure disorders (Scott and Robb Smith 1939) More recently Farquhar and Claireaux (1952) described the occurrence of fatal hæmophagocytic reticulosis in two siblings Macrophages containing erythrocytes were conspicuous in their spleens lymph nodes and bone marrow

HÆMOLYTIC ANÆMIA IN LEUKÆMIA AND RETICULOSARCOMA

The possible role of hæmolysis as a factor in the pathogenesis of anæmia in leukæmia was discussed by Hirschfeld as long ago as 1906 Later Brill (1924) Klima (1934–35) and Jaffé (1935) described patients in whom they thought hæmolysis was occurring Barker (1938) determined the urobilinogen excretion in the urine and fæces of nine patients with leukæmia and found that in two of them suffering from myeloid leukæmia the amount of fæcal pigment was definitely raised their reticulocyte counts were 7.6% and 6.2% respectively More recently Collins and Rose (1948) concluded that hæmolysis was an unimportant factor in the anæmia of leukæmia they considered that blood loss by hæmorrhage and interference with erythropoiesis were more important

The recent use of quantitative methods for the study of the life span of erythrocytes after transfusion has provided conclusive evidence of the impaired survival of transfused normal erythro



FIG 84 Photomicrograph of a blood film of a patient suffering from carcinomatosis and hæmolytic anæmia (Case 23) × 700

was the serum bilirubin concentration definitely above the normal range

Brown Elliott and Young (1951) demonstrated that the survival of transfused normal erythrocytes was impaired (mean cell life 18 to 40 days) in three out of four patients with lymphatic leukæmia and Sheets and co-workers (1951) found that elimination was complete in as little as 6 days in one patient with chronic lymphatic leukæmia

Ross Crockett and Emerson (1951) studied the fate of normal erythrocytes transfused to ten patients suffering from leukæmia or malignant lymphomata as well as the patients fæcal urobilinogen excretions The normal corpuscles were eliminated at two to three times the normal rate in each case but only in one patient were there signs of overt hæmolytic anæmia

Berlin Lawrence and Lee (1951) using ^{14}C tagged glycine reported a normal erythrocyte life span in a patient with chronic lymphatic leukæmia in remission and a minor degree of impaired survival (71 and 76 days) in two patients with chronic myeloid leukæmia

Schwartz and Critchlow (1952) in a review of erythræmic myelosis (di Guglielmo s disease) concluded that increased erythrocyte destruction probably played a part in the causation of the anæmia

Hæmolysis in Myelosclerosis An increased rate of blood destruction is probably always present to a greater or less extent in myelosclerosis and in some patients this may be so marked as to defeat palliative attempts at treatment by blood transfusion In some patients auto antibody formation seems to play a part in bringing about the blood destruction (Rosenfield Vogel and Rosenthal 1951 Hennemann Kunz and Gillert 1953)

Hæmolysis in Polycythæmia Recent studies suggest that a latent hæmolytic element may be detected in polycythæmia vera Lawrence Berlin and Huff (1953) studied the appearance and disappearance of tagged hæmin in the blood stream of five patients to whom ^{14}C labelled glycine had been administered in each instance it seemed that short lived erythrocytes were produced as well as some which survived for a normal length of time In polycythæmia developing in association with myelosclerosis there may be clinical evidence of increased hæmolysis as in the patient reported by Rau Pulvertaft and Humble (1946)

Laboratory Findings in Leukæmia and Myelosclerosis Accompanied by Increased Hæmolysis

The blood picture in those patients who develop latent or overt hæmolytic anæmia is often dominated by the underlying condition from which the patient is suffering Nevertheless signs suggestive *of erythrocyte regeneration and hæmolytic anæmia* may often be observed These include high reticulocyte counts normo

cytes in some cases of chronic leukæmia Moreover it is now well recognized that overt hæmolytic anæmia may not uncommonly develop in the course of chronic leukæmia especially the lymphatic type and sometimes accompany or even precede reticulosarcoma

Haden (1939) described a patient suffering from a hæmolytic anæmia not benefited by splenectomy who six months later developed an obvious chronic lymphatic leukæmia and Singer and Dameshek (1941) referred to two further patients with lymphatic leukæmia and another who suffered from lymphosarcoma who also developed a hæmolytic anæmia Davis (1944) described a patient with acute leukæmia (possibly erythromyelosis) in whom there appeared to be good evidence of hæmolysis and Aubert and Brendemoen (1949) reported a patient in whom a hæmolytic anæmia was associated with an abdominal tumour which at autopsy was found to be a lymphoblastoma Marchal and Duhamel (1950) referred to five cases of leukæmia with hæmolytic anæmia three being myeloid and two lymphatic in type

Jonsson Hansen Pruss and Rundles (1950) described the history of a patient who suffered from myeloid leukæmia and in whom excessive hæmolysis was greatly reduced by splenectomy and Stats (1950) referred to several patients with acute leukæmia in whom transfused normal blood appeared to be destroyed at several times the normal rate Hagen and Watson (1951) referred to six patients with chronic lymphatic leukæmia and patients suffering from myeloid leukæmia and reticulo endotheliosis respectively all of whom had developed an accompanying hæmolytic anæmia Four of these patients all with chronic lymphatic leukæmia were substantially benefited by splenectomy Dameshek Rosenthal and Schwartz (1951) described three more patients with lymphosarcoma or lymphatic leukæmia and hæmolytic anæmia

The work of Berlin (1951) suggests that a latent hæmolytic syndrome is present in many cases of chronic leukæmia He studied fifteen patients with myeloid leukæmia and nine patients with the lymphatic type Transfusing his patients with normal blood and estimating the survival of the normal corpuscles by the Ashby method he found a rapid elimination of the normal cells in twelve out of fifteen patients with myeloid leukæmia and in five out of nine patients with lymphatic leukæmia In seven of the patients (of both groups) the rate of elimination was markedly increased 50% of the transfused cells being eliminated within ten days or less Most of these patients had raised reticulocyte counts the highest observed figures ranging from 2 4 to 9 4% in the patients of the myeloid group and from 0 8 to 12 2% in the patients of the lymphatic group Erythrocyte osmotic fragility was definitely increased in two patients with myeloid leukæmia and in one patient with lymphatic leukæmia Berlin (1951) made the additional point that in only three out of 24 cases of leukæmia

the γ globulin type is obscure they appear to be similar to those seen occasionally in other chronic diseases such as uræmia rheumatoid arthritis sarcoid etc in which abnormalities in the plasma proteins are commonly found

Pathogenesis of the Increased Hæmolysis in Lymphadenoma Leukæmia and Reticulosarcoma etc

As already mentioned in many of the cases of leukæmia lymphadenoma or reticulosarcoma in which there are signs of excessive hæmolysis in addition to the primary disease satisfactory evidence of the formation of auto antibodies is not forthcoming In these cases some other explanation for the hæmolysis must be sought Unfortunately little is known of the processes involved some possible mechanisms are considered below (The way in which auto antibodies may bring about hæmolysis *in vivo* has already been discussed on p 299)

Possible Hæmolytic Effect of Metabolites There is clinical evidence that the severity of a patient s anæmia often varies with the fluctuations in the intensity of the primary disease process itself In lymphadenoma for instance the patients usually become more anæmic during the febrile phases of a cyclic pyrexia (Fig 82) In cases of lymphadenoma too hæmolytic anæmia seems generally associated with massive infiltrations of the spleen and bone marrow It is a possible hypothesis that metabolites injurious to the patients erythrocytes are generated by growing lymphadenomatous (or reticulosarcomatous) tissue and that these metabolities bring about lysis in places where the erythrocytes are brought into intimate contact with the patho logical tissue as may happen when the spleen and bone marrow are infiltrated

Evidence for this hypothesis may be found in some cases if the distribution of hæmosiderin in the pathological tissue is studied In Case 19 for instance each nodule of lymphadenomatous tissue in the spleen was surrounded by a striking cuff of iron containing pigment (Fig 83) In this patient it seems certain that the hæmosiderin was being derived from erythrocytes destroyed in close proximity to lymphadenomatous nodules A very similar appearance is illustrated in Davidson s (1932) paper (his Case 2)

Erythrophagocytosis Another mechanism of erythrocyte destruc tion which may be important is erythrophagocytosis but whether the hyperplastic pathological cells are capable of phagocyto ing normal cells unsensitized by auto antibodies remains uncertain Be that as it may erythrophagocytosis is often a very striking

blastæmia spherocytosis—which is more or less obvious in most cases of myelosclerosis and rather less obvious in chronic myeloid leukæmia—and a moderate or marked degree of polychromasia and punctate basophilia The erythrocyte osmotic fragility is often increased to some extent and reflects the degree of sphero cytosis The plasma bilirubin concentration may be above the *normal range but this is by no means invariable*

Serology in Hæmolytic Anæmia Associated with Lymphadenoma Leukæmia and Reticulosarcoma etc

It is only recently that reports have begun to appear on serological studies in cases of secondary hæmolytic anæmia In some cases evidence of auto immunization has been found with the patients erythrocytes giving positive antiglobulin tests (Trinick 1949 Jordan and Dingle 1949 Rosenfield Vogel and Rosenthal 1951 Willcox 1952 Craig Waterhouse and Young 1952) *in some cases too abnormal antibodies* have been demonstrated in the patients sera (Dameshek Rosenthal and Schwartz 1951 Sulzer 1952 Paraf and Dausset 1952 Hennemann Kunz and Gillert 1953) Two hitherto unpublished cases in which there was evidence of auto immunization are described on pp 336 and 337 respectively It is interesting to note that the type of antibody developed was different in the two cases in Case 20 it was of the warm type whilst in Case 21 it was of the cold type The antibodies could not be distinguished by *in vitro* tests from the auto antibodies of idiopathic cases

The frequency with which signs of auto immunization may be detected in patients suffering from leukæmia reticulosis or reticulosarcoma associated with hæmolytic anæmia is probably greater than the rather scanty reports in the literature suggest It seems certain however that auto antibodies will not be found *in all patients in whom there is good evidence of excessive hæmo* lysis For example in Case 19 a patient who died of lymph adenoma and hæmolytic anæmia the direct antiglobulin test was negative and it was also negative in a personally studied patient *suffering from myeloid leukæmia (with pseudo Pelger* leuco cytes) in whom there was probably an excessive rate of hæmolysis (Darte Dacie and McSorley 1954) On the other hand weakly positive direct antiglobulin reactions may be observed in certain cases of *leukæmia* and in myelosclerosis and *lymphadenoma* without there being necessarily overt hæmolysis *in vivo*

The significance of these reactions which do not appear to be of

to the North Middlesex Hospital under the care of Dr D C Ferriman where he was treated with X ray therapy and blood transfusions The latter became of less and less value and in 1950 the direct antiglobulin reaction was found to be positive and difficulty was also experienced in finding compatible blood with which to transfuse him He was admitted to Hammersmith Hospital in March 1952 for further investigation

Physical Examination On admission he was seen to be a pale but well nourished elderly man He was slightly jaundiced Examination of his cardiovascular respiratory and nervous systems revealed nothing remarkable His liver was palpable 5 cm below the costal margin and the spleen was also palpable to the same extent Enlarged lymph nodes varying in size from 1 to 3 cm in diameter were present in the cervical axillary supratrochlear and inguinal regions

Laboratory Investigations The erythrocyte count averaged 1 350 000 cells per c mm with 6 5 g hæmoglobin per 100 ml the M C V was 140 c μ and the reticulocyte count 31%. The total leucocyte count averaged 9 500 cells per c mm 64% being small lymphocytes Examination of stained peripheral blood films showed considerable anisocytosis polychromasia and spherocytosis The serum bilirubin concentration was 1 5 mg per 100 ml Sternal puncture yielded a cellular marrow 74% of the nucleated cells being mature lymphocytes

Serology (Table 2C) The patient s blood group was B CDe/cde The direct antiglobulin test was positive the antibody behaving as if it were a γ globulin His serum contained free antibody also of γ globulin type which appeared to be capable of sensitizing erythrocytes of all blood groups Absorption experiments showed however that this antibody consisted of three components anti E (an immune iso antibody) and two auto antibodies (anti e and a non specific one) (Dacie and Cutbush 1954 Case 3) The Wassermann and Kahn tests were negative

Further Progress He was transfused with group O Rh negative blood its survival being followed by the Ashby method This showed that 50% of the transfused erythrocytes had been eliminated by about 7 days after the transfusion (Fig 86 p 341) Three days after the start of this survival study he was given a course of A C T H 25 mg being administered intramuscularly four times a day for eleven days The strength of the direct antiglobulin test the rate of elimination of the transfused erythrocytes and the total of circulating lymphocytes were not significantly affected

Summary A case of chronic lymphatic leukæmia associated with acquired hæmolytic anæmia of the warm antibody type No response to A C T H therapy (However the dose of 1 1 g in eleven days may have been too small)

Case Report Acquired Hæmolytic Anæmia (Auto antibody Type) Associated with Reticulosarcoma

Case 4 The patient (L H) was a woman aged 70 years who had been in good health until the summer of 1951 when she gradually developed increasing weakness and dyspnœa In December 1951 she was admitted to Addenbrooke s Hospital Cambridge under the care of Dr A P Dick Acquired hæmolytic anæmia was diagnosed and she was treated with A C T H She responded excellently but in February

feature in the vicinity of reticulosarcomatous tissue when it invades lymph nodes (e g Case 21)

Effect of Splenomegaly It seems probable that the mere size of a pathological spleen is a factor of potential importance in bringing about increased blood destruction Berlin (1951) for instance found in cases of chronic leukæmia that the survival time of transfused normal blood was short in most patients with marked splenomegaly but relatively normal in patients with no enlargement or only moderate enlargement of the spleen It is probable that if for any reason the spleen functions as a hæmolytic organ then the more splenic tissue there is the greater will be the blood destruction

Erythrocyte Abnormalities In myelosclerosis and to a lesser extent in leukæmia anisocytosis and poikilocytosis may be marked features in peripheral blood films The cause of the marked variation in cell size and shape is obscure nevertheless it is probably a type of abnormality which is associated with a diminished erythrocyte life span An appreciable degree of spherocytosis may be seen in some cases and is also probably associated with a reduced erythrocyte survival Whether or not the spherocytosis is the result of the splenomegaly perhaps due to an abnormal degree of vascular stasis in the pathological spleen or whether it is a manifestation of an acquired intrinsic erythrocyte defect determined by the patient s underlying disease remains uncertain Spherocytosis of course may be due to the effects of auto antibodies as in cases of acquired hæmolytic anæmia of the idiopathic auto antibody type However this cannot be the whole explanation for it may sometimes be conspicuous in cases in which auto antibodies cannot be demonstrated as in the patient reported by Darte Dacie and McSorley (1954)

Source of Auto antibodies As has been referred to on p 298 it seems likely that pathological lympho reticular tissue may itself be a source of auto antibodies in some patients Aubert and Brendemoen (1949) for instance demonstrated cold agglutinins in warm saline washings of tumour tissue and a similar observation has been made more recently by Wiener Gordon and Gallop (1953)

***Case Report** Acquired Hæmolytic Anæmia (Auto antibody Type) Associated with Chronic Lymphatic Leukæmia*

Case 20 The patient (A F) was a man aged 71 years who gave a history of anæmia and general weakness which first became noticeable in 1942 He never recovered completely and in 1949 he was discovered to be suffering from chronic lymphatic leukæmia He was then admitted

1952 she relapsed From March to May 1952 she received further A C T H her anæmia again remitted and she continued treatment as an out patient She was admitted to Hammersmith Hospital in July 1952

Physical Examination She was found to be a pale perceptibly jaundiced but well nourished elderly woman Her cardiovascular respiratory and nervous systems were normal for her age Her liver was

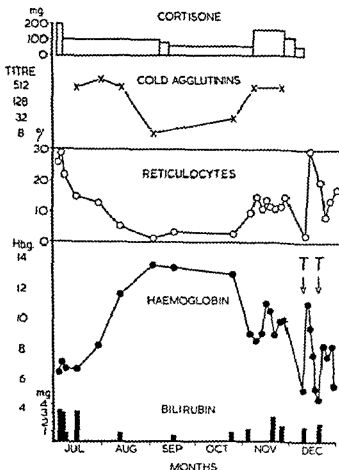


FIG 84 Hæmatological observations on a patient suffering from chronic hæmolytic anæmia of the cold antibody type associated with a reticulosarcoma after treatment by cortisone and blood transfusions (T) (Case 21)

TABLE 26 *Serological data on three patients suffering from secondary acquired hæmolytic anæmia In Case 20 the auto antibodies were of the warm type (anti e and non specific) in Case 21 the auto antibodies were of the cold type and appeared to be non specific*

C se number a d cl ni l state cf pati nt	Dı ct tl gl bulin reaction	Aggl t tlo f orı al erythro ytes (Titre)		I direct ntiglob lin ction		Hæmoly ls f normal ythr ytes pH 6 5		Hæmoly s cf P N H erythro ytes pH 8 0		Agglut nation cf trypsiniz d norm l erythrocytes (Titre)	Hær ly cf tryp inize l normal e ythroc tes pH 8 0 (T t e)
		2 C	3 C	37 C	0 C	37 C	0 C	3 C	0 C	3 C	3 C
19 (Hæmolytic anæmia and lymph adenoma)	–	4	–	–	–	–	–	–	–	–	–
20 (Hæmolytic anæmia and chronic lymphatic leukæmia)	++	16	–	+	+	–	–	–	–	64	–
21 (Hæmolytic anæmia and reticulo sarcoma)	+	512	–	+ *	+++ *	+	+++			8	8

Denotes no observation
* Reactions positive at pH 6 5 negative at pH 8 0 The reactions were negative using serum heated at 56 C for 30 minutes

ment following transfusion was however not sustained her condition steadily deteriorated and she died on December 30th 195_

Postmortem Examination The main macroscopic features were as follows marked auto agglutination of the blood thrush breast myocardium œdema of the lungs with antemortem thrombi in branches of the pulmonary arteries gross enlargement of the spleen (1 650 g) the pulp of which was stippled with small white nodules generalized enlargement of cervical thoracic and abdominal lymph nodes (up to 3 cm in diameter) and a patchy infiltration of the vertebral sternal and femoral marrow with white nodules

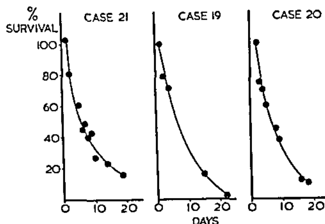


FIG 86 The survival of normal erythrocytes after transfusion to patients suffering from hæmolytic anæmia and lymphadenoma (Case 19) chronic lymphatic leukæmia (Case 20) and reticulo sarcoma (Case 21) respectively

Histology Sections of lymph nodes and spleen suggested that the neoplastic tissue was a reticulosarcoma arising from the lymph follicles Erythrophagocytosis was conspicuous in the bone marrow and in the lymph sinuses of the neoplastic lymph nodes

Summary A case of acquired hæmolytic anæmia (cold auto-antibody type) associated with widespread reticulosarcoma A C T H and cortisone therapy *resulted in temporary clinical and hæmatological* remissions

Treatment of Hæmolytic Anæmia Associated with Lymphadenoma Leukæmia and Reticulosarcoma etc

In addition to supportive measures such as blood transfusions and measures directed against the primary disease such as X ray or nitrogen mustard therapy A C T H and/or cortisone have been administered and splenectomy carried out in attempts to arrest the hæmolytic process

palpable 5 cm below the costal margin and her spleen reached the umbilicus A few slightly enlarged lymph nodes were palpated in the groins and left axilla Her skin was free from purpura but a small hæmorrhage was noted in the right ocular fundus Her urine contained an excess of urobilinogen but no bile

Laboratory Findings On admission her erythrocyte count was 1 600 000 cells per c mm with 6 6 g hæmoglobin per 100 ml the M C V was 155 c μ and the total leucocyte count 5 000 per c mm with 64% neutrophils the platelet count was 245 000 per c mm Examination of a stained blood film revealed macrocytosis and a moderate degree of anisocytosis polychromasia was conspicuous and there was slight spherocytosis Occasional normoblasts were present The erythrocyte osmotic fragility was moderately increased hæmolysis commenced in 0 60% NaCl the M C F being 0 455% NaCl The serum bilirubin concentration was 3 3 mg per 100 ml

Serology (Table 26) The patient s blood group was OM Rh positive The direct antiglobulin test was positive the reaction being of the cold antibody type The cold agglutinin titre using normal group-O corpuscles was 512 at 2 C 64 at 22 C and 4 at 25 C Trypsinized normal corpuscles were agglutinated to a titre of 1 024 at 20 C they were lysed to a titre of 32 At 37 C the hæmolysin titre was 8 Her serum caused marked lysis of normal corpuscles at 20 C but only when acidified to pH 6 5 to 7 0 a small amount of lysis took place at 37 C The indirect antiglobulin test was strongly positive at 20 C but only if the cells were sensitized in acidified serum (pH 6 5) There was also definite but weaker sensitization at 37 C using acidified serum Sensitization both at 20 C and at 37 C was abolished by previously heating the serum at 56 C for 30 minutes These reactions were those of a cold antibody the activity of which extended up to 37 C

Further Progress The patient was treated with cortisone 200 mg a day by mouth later the dose was reduced to 100 mg A good clinical and hæmatological remission resulted (see Fig 85) The cold agglutinin titre fell to within the normal range and the direct antiglobulin test although still positive became weaker The daily dose of cortisone was then further reduced to 50 mg The patient however soon relapsed only to improve once more when the dose was doubled

In November 1952 a mass of enlarged lymph nodes was found to have developed in the left supraclavicular region The small nodes in the axillæ and groins and her spleen had not however increased in size Biopsy of a supraclavicular node revealed the presence of reticulo sarcoma A sternal puncture was also done at this time The marrow was hyperplastic and predominantly erythropoietic it did not contain any neoplastic cells

In view of the malignant nodes in her neck cortisone therapy was suspended and X ray therapy in small doses was directed to the enlarged cervical lymph nodes The patient was transfused on December 11th 1952 with the packed cells from two pints of group OM Rh positive blood and also with the cells from one pint of group ON Rh positive blood The survival of the latter was followed by the Ashby method using an anti M agglutinating serum about 50% of the transfused erythrocytes had been eliminated by the 5th day (Fig 86) Improve-

Singer and Dameshek (1941) reported improvement for five months in a patient suffering from lymphadenoma and hæmolytic anæmia Stats Rosenthal and Wasserman (1947) referred to two patients in one there was no improvement in the other the operation was followed by remission of her anæmia until her death seven months later

Evans and Doan (1951) referred to seven patients with giant follicle lymphoma who underwent splenectomy All three of the authors cases were relieved of their hæmolytic anæmia and remained well for seven years one year and six months respectively

Berlin (1951) reviewed the literature on splenectomy in leukæmia Early reports were not encouraging for although the immediate opera tive mortality was not unduly high most patients seemed to derive little benefit However Ferrata and Fieschi (1939) concluded that in patients with severe hæmolytic anæmia and thrombocytopenia without myeloblastic proliferation in the bone marrow there was a special indication for splenectomy

Other authors such as Gasser (1946) have considered splenectomy to be inadvisable on the grounds that the operation removed an organ thought to exert a humoral inhibitory effect on the bone marrow However Jonsson Hansen Pruss and Rundles (1950) described a patient with chronic myeloid leukæmia in whom splenectomy was remarkably successful During the preceding 11 months this patient had 49 trans fusions after splenectomy the hæmolysis subsided and she received no transfusions during the following 30 months

Hagen and Watson (1951) reported on the results of splenectomy in eight patients with leukæmia and more or less pronounced hæmolytic anæmia Seven of the eight patients were benefited as the result of the operation and four patients with chronic lymphatic leukæmia improved dramatically

Berlin s (1951) observations were based on a study of seven patients six with myeloid leukæmia and one with chronic lym phatic leukæmia In most cases erythrocyte survival studies were carried out before and after splenectomy Two of the patients underwent satisfactory remissions and remained in good health for almost two years after the operation In the others the results were less satisfactory in each case there was an improvement or restoration to normal of the survival of normal transfused erythro cytes but the patients general condition deteriorated for one reason or another Berlin concluded (1) that splenectomy should only be undertaken in cases where there was clear evidence of hyperhæmolysis and (2) that the operation should be performed as early as possible in the course of the disease He also considered that the leukæmia should be brought into remission if possible by chemotherapy or X radiation before the operation was attempted Berlin reviewed the evidence as to whether or not splenectomy *increased* the chances of a fatal myeloblastic crisis and decided that this was inconclusive

Results with A C T H and Cortisone Dameshek Rosenthal and Schwartz (1951) described the effects of A C T H therapy in three cases Two patients with lymphosarcoma were greatly benefited *in both there was a spontaneous rise in hæmoglobin* and a fall in serum bilirubin and in the titre of abnormal agglutinins the malignant process regressed However the direct antiglobulin reaction remained positive and relapse followed cessation of therapy A third patient suffering from chronic lymphatic leukæmia reacted less favourably

Davis and co workers (1952) treated a patient suffering from reticulosarcoma and hæmolytic anæmia with 200 mg of cortisone a day for 27 days Minor increases in the reticulocyte count and *in the total erythrocyte count followed but the anæmia increased* in severity as soon as the cortisone was withdrawn The direct antiglobulin reaction was negative throughout

Of the two cases of this type studied by the author one (Case 20) an elderly man with chronic lymphatic leukæmia and hæmolytic anæmia of the auto antibody type was not benefited by 100 mg of A C T H a day, the other (Case 21) an elderly woman with reticulosarcoma and hæmolytic anæmia derived considerable benefit

Effect of Radiotherapy *There seems to* be little reliable information on the effects of radiotherapy on hæmolysis in the types of hæmolytic anæmia now under discussion

Klima (1934–5) described a patient with chronic lymphatic leukæmia whose anæmia was greatly alleviated as the result of X ray therapy the patient s jaundice diminished and the reticulocyte count fell from 35 to 6 4%

The patient suffering from lymphadenoma and hæmolytic anæmia with spherocytosis described as Case 5 by Stats Rosenthal and Wasserman (1947) also benefited from X ray therapy the lymph node enlargement and the hæmolytic anæmia both subsided Stats and his colleagues referred to other published accounts in which the effects of radiotherapy seemed less conclusive and also mentioned the possibility that X radiation may have precipitated hæmolytic episodes in some cases (see also Singer and Dameshek 1941 and Marchal and Duhamel 1950) Nevertheless despite the possibility of exacerbations *in hæmolysis it seems to the author reasonable to treat the primary* disease by every available means and not to be deterred just because the patient has a hæmolytic anæmia

Effect of Splenectomy Splenectomy has been carried out in a number of patients suffering from reticuloses or leukæmia with hæmolytic anæmia The results have been variable usually there has been some temporary improvement occasionally a dramatic remission has ensued

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Fisher Welch and Dameshek (1952) have also summarized the literature on the results of splenectomy in cases of leukæmia and leuko sarcoma In addition they reported the results of the operation in eighteen personally studied patients who suffered from hæmolytic anæmia pancytopenia or thrombocytopenia in addition to their primary disease Eight of the eighteen patients derived sustained benefit from splenectomy Three of the patients with hæmolytic anæmia and chronic lymphatic leukæmia were considered to have been improved in health for one month three months and 50 months respectively after splenectomy one patient with monocytic leukæmia was improved for three months and one patient with giant follicle lymphoma for 4? months Two patients with myeloid leukæmia and lymphadenoma respectively derived no benefit

HÆMOLYTIC ANÆMIA IN ASSOCIATION WITH CARCINOMATOSIS

Anæmia is a common complication of cancer However according to Shen and Homburger (1951) this is rarely due to hæmolysis for in only three out of a total of 116 anæmic cases investigated by them was there clear evidence of hæmolytic anæmia The criteria for the diagnosis of hæmolytic anæmia used by Shen and Homburger were either a positive Coombs test or an increase in the osmotic or mechanical fragility of the red cells together with reticulocytosis hyperbilirubinemia and an increased output of blood pigments It seems reasonable to suppose that if the criterion for diagnosis had been the finding of an impairment in the survival of transfused normal erythrocytes the percentage of patients in whom there was evidence of hæmolysis would have been considerably increased

Overt Hæmolytic Anæmia

There are in the literature a number of descriptions of severe anæmia of apparently hæmolytic type associated with widely disseminated carcinoma Usually the bone marrow has been extensively infiltrated Typical case histories have been published by Waugh (1936) Caroli and Lavergne (1937) Lucey (1939) Holmes and McCall (1940) Davis (1944) Stats Rosenthal and Wasserman (1947) and Hogeman (1953) According to Paraf and Dausset (1952) the stomach is the commonest site of the primary tumour In some instances a rather rapid onset of severe anæmia perhaps with pyrexia was the first sign of the patient s illness in other patients the anæmia was the first sign that the disease had become disseminated following previous excision of the primary tumour Hæmolytic anæmia in carcinomatosis is pro bably not nearly so uncommon as the literature suggests Two

unpublished cases are briefly reported on pp 346-348 (Cases 22 and 23)

Laboratory Findings Anæmia may be severe the hæmoglobin concentration falling to 4 g per 100 ml or less and the erythrocyte count to 1 000 000 cells per c mm or less The reticulocyte count usually ranges between 5 and 20% but may be much higher The M C V lies between 90 and 120 c μ as a rule The total leucocyte count varies usually it is raised and may even exceed 30 000 cells per c mm The majority of the leucocytes are neutrophils almost always 1 to 10% myelocytes are present and occasionally a very few myeloblasts There is usually a moderate degree of thrombocytopenia occasionally this may be severe and lead to a hæmorrhagic diathesis (Frandsen 1949) The erythrocytes characteristically vary considerably in size and shape and there is usually a moderate to marked degree of polychromasia or punctate basophilia Normoblasts are almost invariably present Rounded spherocytes may be seen in some cases in others a variable number of irregularly contracted and distorted corpuscles may be found (Fig 86) Erythrocyte osmotic fragility is often increased (Waugh 1936 Caroli and Lavergne 1937 Lucey 1939 Stats Rosenthal and Wasserman 1947) The plasma bilirubin concentration is usually moderately raised but seldom exceeds 2 mg per 100 ml unless tumour metastases are present in the liver or obstructing the bile ducts The direct antiglobulin test and tests for abnormal antibodies in the serum are usually negative Jordan and Dingle (1949) however reported a positive direct antiglobulin reaction in one case using low dilutions of the antiglobulin serum

Latent Hæmolytic Anæmia

Hæmolysis is probably a factor in the causation of the anæmia of patients with cancer far more frequently and to a far greater extent than was at one time supposed particularly when the bone marrow has been invaded by metastases Sheets Hamilton DeGowin and Janney (1954) found for instance that normal blood transfused to five patients suffering from carcinoma of the cervix breast and rectum and multiple myeloma respectively was eliminated unusually rapidly in a random manner Sheets and co workers also observed in three other patients suffering from carcinoma of the cervix in whom the cell survival was initially normal that an increased rate of hæmolysis appeared to follow within seven to ten days of commencing treatment with X radiation or radium It was calculated that in most patients

Fisher Welch and Dameshek (1952) have also summarized the literature on the results of splenectomy in cases of leukæmia and leukosarcoma In addition they reported the results of the operation in eighteen personally studied patients who suffered from hæmolytic anæmia pancytopenia or thrombocytopenia in addition to their primary disease Eight of the eighteen patients derived sustained benefit from splenectomy Three of the patients with hæmolytic anæmia and chronic lymphatic leukæmia were considered to have been improved in health for one month three months and 50 months respectively after splenectomy one patient with monocytic leukæmia was improved for three months and one patient with giant follicle lymphoma for 4[illegible] months Two patients with myeloid leukæmia and lymphadenoma respectively derived no benefit

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the epigastrium and that later she had had actual epigastric pain and difficulty in swallowing In June 1947 she had been admitted to hospital for investigation without anything positive being found a diagnosis of anxiety neurosis was made When readmitted ten weeks later she was obviously extremely ill Anorexia was marked and epigastric pain severe

Physical Examination The patient was pale and jaundiced A large hard mass palpable in the right upper abdominal quadrant was thought to be an enlarged liver Her urine contained a trace of bile pigment and excess urobilin

Her general condition gradually deteriorated and she died 13 days after admission

Laboratory Observations In June 1947 her blood count was normal On September 17th her hæmoglobin concentration was 11 7 g per 100 ml by September 23rd it had fallen to 8 9 g per 100 ml and the total erythrocyte count had fallen from 3 500 000 to 2 600 000 cells per c mm The reticulocyte count ranged between 11 and 15% the M C V was 103 c μ There were 19 000 leucocytes per c mm 85% of which were neutrophils The plasma bilirubin concentration was 2 9 mg per 100 ml the prompt van den Bergh reaction being negative

Stained blood films were remarkable chiefly for the large number of cells which were distorted contracted and irregularly crenated some of them being almost triangular in shape (Fig 4 p 13) Polychromasia was conspicuous and occasional normoblasts and myelocytes were present The direct antiglobulin reaction was negative

Postmortem Examination The main features were as follows the stomach contained a fungating carcinoma at the cardia 3 cm in diameter The liver weighed 6 250 g and was heavily infiltrated with numerous carcinomatous secondaries many of them being necrotic The abdominal lymph nodes were also infiltrated The spleen weighed 160 g it was dark red in colour but not obviously invaded by growth The bone marrow was hyperplastic and many macroscopic secondaries were present in the vertebræ and femora

Histology Sections showed the tumour to be an adenocarcinoma The spleen was congested with blood and there was considerable siderosis and also some foci of extramedullary hæmopoiesis The bone marrow was heavily infiltrated with tumour cells erythropoietic marrow predominated in areas free from growth

Summary A case of carcinoma of the stomach with widespread metastases particularly in the liver and bone marrow The rapidly progressing anæmia the high reticulocyte count and the distortion of the erythrocytes all suggested that hæmolysis played an important part in the genesis of the anæmia

Case Report Probable Hæmolytic Anæmia Associated with Carcinomatosis (Carcinoma of Stomach)

Case 3 The patient (F K) a woman aged 49 years was admitted to hospital with a history that for the last ten months she had suffered from epigastric pain and vomiting of increasing severity and that recently she had become breathless and weak A diagnosis of inoperable carcinoma of the stomach was made The patient s general condition steadily deteriorated and she died five days after admission

erythrocyte delivery was accelerated in an effort to compensate for the anæmia despite the presence of tumour cells in the bone marrow

Leuco erythroblastic anæmia

The description that has been given of the blood picture in hæmolytic anæmia associated with carcinomatosis is similar to the leuco erythroblastic anæmia of Vaughan (1936) According to Vaughan the salient feature of this anæmia is the presence of normoblasts and immature granulocytes in the peripheral blood of a patient not suffering from leukæmia Vaughan associated this picture with malignant invasion of bone (among other causes) and pointed out that the anæmia might not be severe She attributed the anæmia primarily to dyshæmopoiesis resulting from the presence of actively growing malignant tissue among the bone marrow cells However as her patients with carcinomatosis had reticulocyte counts ranging from 3 8 to 7 1% it is possible that hæmolysis may also have been a factor in the causation of the anæmia

Pathogenesis The cause of the increased hæmolysis in carcinomatosis is unknown It does not seem likely that it is often due to the formation of anti erythrocyte antibodies A possible explanation is that it is brought about by a close contact between the erythrocytes and their precursors and actively growing and possibly necrosing tumour tissue It seems significant that the severest grades of anæmia appear to be found in patients whose marrows are widely infiltrated with new growth It is possible that the abnormalities of erythrocyte morphology e g the contraction and distortion (Figs 4 and 84) and the sphero cytosis are due to a direct effect on the erythrocyte surfaces of toxic products derived from the tumour tissue The same mecha nism may operate in hæmolytic anæmia associated with lymph adenoma and reticulosarcoma It may be significant that products of normal tissue as well as of tumours particularly when autolysing have been shown to be lytic to normal erythro cytes *in vitro* (Cross 1948 1949 Ponder 1951) It is possible too that the hæmolysis that Sheets and co workers (1954) observed after their patients had received X radiation may also be explained by the liberation of toxic products from the irradiated tumour tissue for it is doubtful whether irradiation itself exerts any direct hæmolytic effect (Davis Dole Izzo and Young 1950)

Case Report Probable Hæmolytic Anæmia Associated with Carcinomatosis (Carcinoma of Stomach)

Case 22 The patient (M B) was a married woman aged 48 years She was admitted to hospital in September 1947 giving a history that for the previous five months she had felt a numbing sensation in

the epigastrium and that later she had had actual epigastric pain and difficulty in swallowing. In June 1947 she had been admitted to hospital for investigation without anything positive being found: a diagnosis of anxiety neurosis was made. When readmitted ten weeks later she was obviously extremely ill. Anorexia was marked and epigastric pain severe.

Physical Examination. The patient was pale and jaundiced. A large hard mass palpable in the right upper abdominal quadrant was thought to be an enlarged liver. Her urine contained a trace of bile pigment and excess urobilin.

Her general condition gradually deteriorated and she died 13 days after admission.

Laboratory Observations. In June 1947 her blood count was normal. On September 17th her hæmoglobin concentration was 11.7 g. per 100 ml.; by September 23rd it had fallen to 8.9 g. per 100 ml. and the total erythrocyte count had fallen from 3,500,000 to 2,600,000 cells per c.mm. The reticulocyte count ranged between 11 and 15%; the M.C.V. was 103 c.μ. There were 19,000 leucocytes per c.mm., 85% of which were neutrophils. The plasma bilirubin concentration was 2.9 mg. per 100 ml., the prompt van den Bergh reaction being negative.

Stained blood films were remarkable chiefly for the large number of cells which were distorted, contracted and irregularly crenated, some of them being almost triangular in shape (Fig. 4, p. 13). Polychromasia was conspicuous and occasional normoblasts and myelocytes were present. The direct antiglobulin reaction was negative.

Postmortem Examination. The main features were as follows: the stomach contained a fungating carcinoma at the cardia 3 cm. in diameter. The liver weighed 6,500 g. and was heavily infiltrated with numerous carcinomatous secondaries, many of them being necrotic. The abdominal lymph nodes were also infiltrated. The spleen weighed 160 g.; it was dark red in colour but not obviously invaded by growth. The bone marrow was hyperplastic and many macroscopic secondaries were present in the vertebræ and femora.

Histology. Sections showed the tumour to be an adenocarcinoma. The spleen was congested with blood and there was considerable siderosis and also some foci of extramedullary hæmopoiesis. The bone marrow was heavily infiltrated with tumour cells; erythropoietic marrow predominated in areas free from growth.

Summary. A case of carcinoma of the stomach with widespread metastases particularly in the liver and bone marrow. The rapidly progressing anæmia, the high reticulocyte count and the distortion of the erythrocytes all suggested that hæmolysis played an important part in the genesis of the anæmia.

Case Report. Probable Hæmolytic Anæmia Associated with Carcinomatosis (Carcinoma of Stomach)

Case 23. The patient (F. K.), a woman aged 40 years, was admitted to hospital with a history that for the last ten months she had suffered from epigastric pain and vomiting of increasing severity, and that recently she had become breathless and weak. A diagnosis of inoperable carcinoma of the stomach was made. The patient's general condition steadily deteriorated and she died five days after admission.

Laboratory Observations There were 1 400 000 erythrocytes per c mm and 3 7 g hæmoglobin per 100 ml with 10% reticulocytes 10 000 leucocytes per c mm and 48 000 platelets per c mm The M C V was 86 c μ The plasma bilirubin concentration was 0 9 mg per 100 ml The erythrocyte osmotic fragility was slightly increased lysis commenced in 0 55% NaCl the M C F being 0 42% NaCl

Stained films of her peripheral blood showed marked anisocytosis and polychromasia A striking finding was the presence of many contracted microcytes many of them having irregularly crenated contours some being almost triangular (Fig 84) An occasional myeloblast and 4% myelocytes were present as well as numerous normoblasts (3 600 per c mm)

A sternal bone marrow biopsy showed carcinoma cells in the aspirated material

Postmortem Examination The main abnormal findings were as follows There was a large infiltrating carcinoma occupying the distal one third of the stomach and an extragastric secondary tumour in the lesser omentum The para aortic mediastinal and supraclavicular nodes contained secondary growth The liver and other abdominal viscera appeared free from metastases The spleen weighed 210 g it was congested but likewise appeared to be free from new growth The bone marrow of the vertebræ and sternum was diffusely invaded by carcinoma

Histology Sections showed the tumour to be a rapidly growing mucin secreting adenocarcinoma The spleen was congested and diffusely infiltrated by small groups of malignant cells Erythro phagocytosis was conspicuous and the splenic macrophages were loaded with hæmosiderin

Summary A case of carcinoma of the stomach with multiple secondaries in lymph nodes and in the bone marrow This was accom panied by a severe anæmia of leuco erythroblastic type with evidence of erythrocyte regeneration Many contracted and distorted erythro cytes were present in the peripheral blood

Hæmolytic Anæmia Associated with Ovarian Tumours

There are a few reports of a remarkable type of hæmolytic anæmia developing in association with ovarian tumours Most of the tumours have been dermoids or teratomas

West Watson and Young (1938) described the history of a woman aged 44 who developed a severe hæmolytic anæmia with marked spherocytosis Splenectomy was performed but the hæmolytic process continued unabated Four months later laparotomy undertaken to exclude the presence of splenunculi revealed an ovarian teratoma It was removed and the patient made an uninterrupted recovery

Singer and Dameshek (1941) recorded an almost exactly similar case Their patient was a woman aged 47 years who complained of increasing pallor of about five months duration Examination of her blood showed a severe hæmolytic anæmia with marked microspherocytosis

Splenectomy resulted in some improvement but this was not sustained and the patient needed repeated transfusions Eventually seven months after splenectomy a further laparotomy was undertaken A dermoid cyst of the ovary was found and removed The hæmolytic process then subsided and within four months the blood picture was normal

Jones and Tillman (1945) published another example Their patient was a 35 year old woman who had previously suffered from dermatomyositis Increasing weakness and jaundice led to the diagnosis of hæmolytic anæmia with spherocytosis Clinical examination however showed the presence of a pelvic tumour This was excised and proved to be an ovarian pseudo mucinous cystadenocarcinoma Following its removal the hæmolytic process subsided Splenectomy was not carried out Another possible case was recorded by Lindeboom (1950) His patient however died soon after the removal of a dermoid cyst of the ovary

A further example of this syndrome was reported by Allibone and Collins (1951) The patient was a little girl aged four years and nine months who had been pale for three months and more recently jaundiced Blood examination revealed a severe hæmolytic anæmia with marked spherocytosis Physical examination showed an abdominal tumour which on laparotomy proved to be a cystic teratoma of the ovary The child s blood picture rapidly returned to normal after the operation Her spleen was not removed

Watson s (1939) report is concerned with the occurrence of hæmolytic anæmia in a patient who had an ovarian cyst into which hæmorrhage had occurred In this case however the anæmia was not affected by ovariotomy Slow improvement leading to ultimate recovery followed splenectomy carried out later It is probable therefore that in this instance the ovarian cyst was not related pathogenetically to the anæmia

Pathogenesis Nothing is known as to how and why hæmolytic anæmia develops in certain cases of ovarian tumour It is probably significant that in most of the published cases the tumour was a teratoma or dermoid cyst It seems most likely that the anæmia is due to auto immunization and that this is in some way linked with the presence of the teratoma or dermoid It should be added however that the direct antiglobulin test was negative in the one case in which it was carried out (Allibone and Collins 1951)

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CHAPTER 14

HÆMOLYTIC ANÆMIAS OF DOUBTFUL PATHOGENESIS

In this chapter brief descriptions will be given of a number of types of hæmolytic anæmia of obscure ætiology and pathogenesis. In some cases an allergic mechanism may operate as for example in favism and blackwater fever; in others the hæmolysis appears to be secondary to serious metabolic defects as in uræmia and liver disease. Another type of hæmolytic anæmia is that which is superimposed upon a collagen disease such as disseminated lupus erythematosus while in still other patients there seems to be no explanation for the hæmolysis. The chapter ends with a brief consideration of hæmolytic anæmia due to primary hypersplenism and march hæmoglobinuria.

HÆMOLYTIC ANÆMIAS PROBABLY OF ALLERGIC ORIGIN

Favism

Favism is the name given to a remarkable type of hæmolytic anæmia developing apparently as the result of sensitivity to the broad bean *Vicia faba*. It is found mainly in Sardinia, Sicily and Calabria although occasional examples of the disease have been reported from other areas mostly however in patients of Italian ancestry. In America it has been recorded by Macrae and Ullery (1933), Hutton (1937), Josephs (1944), Lecks (1947), Jacobs (1950) and others. Even in Britain it has been diagnosed occasionally (Diggle 1953). Josephs's and Jacobs's patients were Greek or partly Greek children. Favism is probably not uncommon in Palestine (Robinson 1941) and according to Luisada (1941) the disorder was formerly widely distributed throughout the Mediterranean basin. Favism has a large, mainly Italian literature. Luisada listing over 100 papers published up to 1941. The disease has been reviewed recently by Marcolongo (1953).

Clinical Features (Luisada 1941; Robinson 1941). The salient feature of the disease is the sudden development of an

acute episode of hæmolysis followed by jaundice and often accom panied by hæmoglobinuria Children seem to be most frequently affected and as a rule the attacks last from two to six days The attack can result either from the inhalation of pollen derived from the flowers of the beans or from ingestion of fresh or partially cooked beans When due to inhalation the attack may commence within a matter of minutes after ingestion there is usually a time lag of from five to 24 hours Dizziness nausea vomiting and pyrexia usually herald an attack The episodes vary greatly in intensity hæmolysis may be fulminating or at the other end of the scale the attack may give rise to only the mildest con stitutional symptoms The spleen usually becomes palpable in patients suffering from moderate to severe degrees of hæmolysis

Hæmatology Anæmia may be extremely severe with the erythrocyte count falling below 1 000 000 cells per c mm No noteworthy morphological changes in the erythrocytes have been noticed during the hæmolytic phase During recovery poly chromasia and normoblastæmia are conspicuous the degree of the reticulocytosis being proportional to the severity of the anæmia Osmotic fragility is said to be normal or only slightly increased and spherocytosis does not seem to occur (Luisada 1941 Robinson 1941) The total leucocyte count is raised and counts up to 40 000 per c mm have been observed the majority of the cells being neutrophils Robinson (1941) referred to eosino philia of the bone marrow and peripheral blood he also men tioned three patients in whom there was a deficiency of normo blasts in the marrow at the height of the hæmolytic crisis (see also p 171)

Serology Recent investigations carried out by Marcolongo and his associates on more than 190 cases (Marcolongo 1953) indicate that incomplete warm antibodies may often be demon strable in the patients sera and adsorbed to their erythrocytes at the time of the hæmolytic attacks Thus the direct and indirect antiglobulin tests were found to be positive in more than 80% of cases and antibody could also be demonstrated by means of the bovine albumin plasma albumin and trypsinized cell techniques The tests were observed to be positive in the early hours of an attack and they usually remained positive throughout the whole of the hæmolytic episode Marcolongo (1953) also made the point that antibody was less easily demonstrable and more transient in children and in attacks which were clinically mild

Pathogenesis There are certain predisposing factors First there is apparently a racial predisposition for most cases have

occurred in people of Sardinian extraction, secondly the disease frequently occurs in more than one member of a family (Hutton 1937 Luisada 1941) thirdly there appears to be a personal predisposition for some patients suffer from repeated attacks The ingestion of extremely small amounts of fava beans will precipitate an attack in a sensitive person—even one bean may be sufficient (Luisada 1941) and for Macrae and Ullery's (1933) patient walking through fields of blossoming beans resulted in attacks of unconsciousness on several occasions All the above features point to a constitutional and allergic origin for the hæmolytic attacks and skin testing with extracts of bean flowers pods and beans gives some support for this hypothesis (Luisada 1941) although negative results have been reported also (Lecks 1947 Jacobs 1950) In rabbits too there is evidence that bean extracts may cause shock and hæmoglobinuria the allergic basis of the reactions is however not so obvious as in man (Luisada 1941)

Many beans are now known to contain hæmagglutinin (Renkonen 1948 Boyd and Reguera 1949) and several have been used experimentally to cause hæmolytic anæmia e g the Jack bean *Canavalia* (Ham and Castle 1940) The effect of fava bean extracts on erythrocytes *in vitro* has recently been studied by Creger and Gifford (195?) They found that saline extracts of *Vicia fava* agglutinated human erythrocytes irrespective of their blood groups and also rabbit erythrocytes Agglutination was strongly potentiated by the presence of acacia but not by albumin serum inhibited agglutination Fava bean sensitized cells were not hæmolysed *in vitro* by the addition of fresh human serum complement The exact significance of these observations in relation to clinical attacks of hæmolysis in man awaits elucidation Nevertheless it is noteworthy that these tests have revealed definite interactions *in vitro* between human erythrocytes and bean extracts

Favism is remarkable amongst the acute hæmolytic anæmias because the exciting cause is known It is interesting to reflect that in many of the published case reports of favism the original clinical diagnosis was acute hæmolytic anæmia (? Lederer's type) and that this was only revised often retrospectively when a clear history of the ingestion of beans was forthcoming Robinson (1941) suggested that many of the cases formerly diagnosed in Palestine as blackwater fever which had a peak incidence in the spring time were in reality examples of favism

Treatment Blood transfusion is the treatment of choice in an acute attack and should be given if the patient becomes seriously anæmic Once the patient has been tided over the acute crisis, recovery can be confidently predicted as the hæmolytic

episodes are essentially short lived Anti histamine drugs and A C T H may also be of value (Marcolongo 1953) The most important point once the cause of the hæmolysis has been established is to advise the patient against eating any more of the offending beans The possibility of artificial desensitization was considered by Luisada (1941)

Baghdad Spring Anæmia

Another clinical type of acute hæmolytic anæmia rather similar to favism and also probably allergic in origin was named Baghdad Spring Anæmia by R Lederer (1940–41) Fourteen cases were studied between March and May 1940 All of the patients were boys most being Jews one of them died The attacks were ushered in by abdominal pains and vomiting then anæmia and jaundice quickly developed The spleen was palpable in three instances The urine contained excess urobilin Four of the patients had albuminuria and in one severe case there was hæmoglobinuria Some of the children gave histories of previous attacks at the same time in previous years

Lederer considered that the hæmolysis was due to contact with flowers or young fruits but the exact allergen was not identified Some of the cases may in fact have been examples of favism

Laboratory Observations The erythrocyte counts of most of the children fell sharply to about 1 000 000 cells per c mm The leucocyte count was increased usually to between 18 000 and 30 000 per c mm most of the cells being neutrophils a few myelocytes were present in most instances The erythrocyte osmotic fragility was normal

Acute Hæmolytic Anæmia (?) of Allergic Origin Familial Acute Hæmolytic Anæmia

Bernard (1950a and b) described under the title hemolyse aigue familiale three families in which more than one member suffered from acute hæmolytic anæmia at different times in the first family two brothers were affected in the second a mother and her son and in the third family three small boys All the serological investigations yielded negative results The erythrocyte osmotic fragilities were also normal Bernard concluded that although a diagnosis of favism appeared to be improbable the episodes were likely to be due to an inherited sensitivity to some unknown allergen

Fois (1950) reported a rather similar occurrence A child of two months suffered from a transient acute hæmolytic episode on inquiry into the family history it transpired that the father had suffered from favism and an older sister had died of an acute hæmolytic anæmia with hæmoglobinuria of unknown origin Ingestion or exposure to beans appeared to have nothing to do with the onset of the infant s anæmia

As already referred to in Chapter 7 (p 177) sporadic instances of acute hæmolytic anæmia of short duration and unknown origin

are not uncommon particularly in children In some of these cases of ' Lederer s anæmia the cause of the hæmolysis appears to be the development of auto antibodies in others particularly when the anæmia is of *short duration auto antibodies are not* readily demonstrable In these cases as in the familial cases referred to in the previous paragraphs a sensitivity mechanism appears to be the most likely cause of the hæmolysis

Blackwater Fever

Blackwater fever has been recognized as a serious complication of malaria since the end of the nineteenth century but references to its occurrence can be found in medical writings long before this *(Blackie 1944) Geographically blackwater fever has occurred* chiefly in tropical and subtropical regions of Africa in India Ceylon and the Far East in central America and in Macedonia Racially nearly all the victims have been Europeans The disease is far less frequently met with at the present time than formerly Doubtless this is the result of successful efforts at the eradication of malaria The majority of cases have occurred in the course of infection with *P falciparum* in many instances the actual attack seems to have been precipitated by the taking of quinine or other *plasmodicidal drugs*

Clinically an attack is usually ushered in by a rigor associated with high pyrexia Prostration and vomiting are common accompaniments The urine varies in colour from port wine almost to black and in severe cases oliguria or even anuria may occur On the day following the start of the attack the patient is usually jaundiced *The clinical features are* thus of an acute intravascular hæmolysis associated with general prostration and pyrexia

Hæmatology The erythrocyte count may fall to low levels within a few hours counts as low as 1 000 000 per c mm have been recorded (Blackie 1944) Stained peripheral blood films may reveal the presence of malaria parasites but often they are absent The morphology of the surviving erythrocytes is not strikingly abnormal There may be slight spherocytosis at the *height of the paroxysm (Fairley and Murgatroyd 1940* Fox and Kondi 1943–44) During recovery a reticulocytosis develops dependent upon the severity of the anæmia with a corresponding degree of polychromasia and punctate basophilia in the fixed and stained blood film Normoblasts and myelocytes may be present in the peripheral blood in small numbers at the *height of the* hæmolysis and during the early recovery phase

Oxyhæmoglobin and methæmalbumin are characteristically

found in the plasma (Fairley 1941) as well as a raised bilirubin concentration the latter persisting well into the recovery phase Erythrocyte osmotic fragility is usually normal but the sensitivity to lysis by lysolecithin may be increased (Foy and Kondi 1943–44 Foy 1948)

The urine characteristically contains variable amounts of oxyhæmoglobin methæmoglobin urobilinogen albumin and casts

Serology The direct antiglobulin test was negative in the few cases in which the reaction was carried out (Foy 1948) Similarly attempts to demonstrate abnormal antibodies in the serum seem to have been unsuccessful (see also under *Pathogenesis*)

Pathology In fatal cases the *spleen* is always found to be enlarged and engorged with blood The littoral cells and reticulum cells of the pulp are hyperplastic and prominent and there is usually evidence of erythrophagocytosis Malarial pigment may also be present The *liver* appears congested with blood and engorged with bile pigment Areas of necrosis may be visible Malarial pigment may be seen in Kupffer cells The *kidneys* show a variable degree of lower nephron nephrosis with pigment cast formation

Ætiology and Pathogenesis

The cause of blackwater fever still remains a baffling problem As a rule it occurs during the course of malignant tertian malaria affecting a relatively non immune (i e European) population often it appears to be precipitated by the taking of quinine or atebrin (Fairley and Murgatroyd 1940 Foy and Kondi 1937) There is no evidence that special hæmolytic strains of the parasites are involved (Foy Kondi and Moumjidis 1941–42)

Morphological and serological studies have failed as yet to demonstrate the mechanism of hæmolysis In man at least the damaged corpuscles do not seem to pass through a markedly spherocytic phase prior to their destruction However in monkeys heavily infected with *P knowlesi* Shen Fleming and Castle (1946) have shown that the osmotic and mechanical fragilities of the parasitized corpuscles are substantially increased In blackwater fever in man where the degree of parasitization of the erythrocytes is often negligible it appears highly improbable that the parasites are directly responsible for the hæmolysis It is known moreover that normal as well as the patient s erythrocytes undergo rapid hæmolysis in the patient s circulation during the actual paroxysm and that the patient s cells are destroyed rapidly in a normal recipient (Foy Kondi and Moumjidis 1941–42

Fox Kondi Rebelo and Soeiro 1944–45 Mollison 1947) The possibility that the patient s blood is deficient in an antihæmolytic factor which is normally present (Maegraith Martin and Findlay 1943) awaits confirmation

It has been suggested (Gear 1945–46) that hæmolysis is determined by the development of auto antibodies as the result of the patient s erythrocytes developing antigenic properties due to some alteration produced by the malarial infection and/or the anti malarial drug This suggestion analogous to that postulated to explain the occasional incidence of hæmolytic anæmia due to drugs such as the sulphonamides (see p 396) is attractive but purely hypothetical for the antibodies if they exist have not yet been demonstrated Nevertheless it seems possible that the hæmolytic mechanism may be similar to that in the transient hæmolytic anæmias due to drug idiosyncrasies or unknown causes in all of which the conventional tests for antibodies seem to be negative It is possible that blackwater fever and the other types of hæmolytic anæmia that have just been mentioned are sensitivity reactions the union between allergen and antibody taking place with disastrous results at the surface of the erythrocytes

HÆMOLYTIC ANÆMIA ASSOCIATED WITH DISEASES OF THE LIVER

The significance of concurrent liver disease in acquired hæmolytic anæmia is often difficult to assess In some instances it seems probable that the liver disease develops as a consequence of the hæmolytic process For example it is possible that liver cell damage may be due to the severity of the patient s anæmia or to autohæmagglutination in the liver sinuses In other cases the liver damage may be secondary to the formation of pigment gallstones or even may follow serum hepatitis caused by a previous blood transfusion

The actual diagnosis of liver disease may be difficult unless biopsy is carried out The so called chemical tests of liver function cannot be relied on in the presence of hæmolytic anæmia for changes in the serum globulins are frequently encountered in hæmolytic anæmia quite irrespective of the presence or absence of liver disease

There is however no doubt that increased blood destruction may be superimposed upon pre existing liver disease Even so this may be missed unless looked for carefully Minor degrees of excessive hæmolysis are probably not uncommon Fellinger and

Klima (1933–34) concluded that blood destruction was the most important cause of the anæmia which frequently accompanies chronic cirrhosis of the liver More recently Chaplin and Mollison (1953) found by means of transfusion studies that the rate of erythrocyte destruction was from two to five times the normal in five patients suffering from cirrhosis The hæmoglobin concentrations of their patients (before transfusion) ranged from 9 2 to 11 2 g per 100 ml and their reticulocyte counts from 2 0 to 5 8% There are moreover in the literature a small number of case reports of patients with liver disease in whom hæmolytic anæmia was easily recognized and sometimes even came to dominate the clinical picture

Hijmans van den Bergh and Kanerling (1935) described a patient who died of a hæmolytic anæmia associated with intravascular hæmolysis At postmortem advanced cirrhosis of the liver was the most important finding Watson (1937) referred to seven patients with clinical evidence pointing to cirrhosis of the liver and increased blood destruction the fæcal urobilinogen excretions varied from 319 to 1 257 mg per day and in three cases the reticulocyte counts were reported as 7 12 and 15 % respectively Watson (1939) referred to these cases again and described another patient The eight patients were encountered in a group of 59 patients thirty eight of whom were believed to have cirrhosis of the liver and twenty one catarrhal jaundice

Davidson and Fullerton (1938) reported the finding of extensive portal cirrhosis in a patient who died of a severe acquired hæmolytic anæmia Voit (1948) referred to several cases reported in the German literature in which a change from chronic hepatitis to hæmolytic anæmia had been observed and Coleman (1948) described two patients with severe anæmia apparently hæmolytic in type in whom cirrhosis of the liver was found at postmortem Other patients were described by Hahn and Luttgens (1949) and by Cattan and co workers (1952) More recently Hyman and Southworth (1951) described two patients with cirrhosis proved by biopsy and hæmolytic anæmia and referred to an earlier patient in whom the findings were similar

Other patients with hæmolytic anæmia and evidence of liver cell damage but not of chronic liver disease were reported by Lovibond (1935)—at autopsy the patient s liver showed the changes of acute yellow atrophy by Farrar Burnett and Steigman (1940)—at biopsy at the time of splenectomy for an acute hæmolytic crisis the liver cells showed granular and fatty degeneration by Singer and Dameshek (1941)—liver biopsy showed moderate fibrosis and by Stacey (1946)—at autopsy the patient s liver cells were reported as showing much cloudy swelling and fatty degeneration Hyman and Southworth (1951) also referred to five additional patients in whom liver biopsy showed various combinations of diffuse swelling of the liver cells focal necrosis central congestion and in most cases hæmosiderosis

Pathogenesis It is interesting to note that when *acute*

liver cell damage has been reported in patients whose hæmolytic anæmia dominated the clinical picture this was either found at autopsy in patients who had died of their disease or was seen in liver biopsies taken when the patient was seriously ill It is probable that this type of damage is due to the effects of anæmia and/or autohæmagglutination

The nature of the apparent association between chronic liver disease and increased hæmolysis is uncertain Possibly in some cases it is the result of the circulation of hæmotoxic substances of endogenous origin due to impairment of the liver s detoxicating function In other cases it is possible that qualitatively abnormal globulins capable of sensitizing erythrocytes are formed concurrently with the development or accumulation in the blood of increased amounts of globulins Autohæmagglutination was conspicuous in the patient described by Hahn and Luttgens (1949) and it is interesting to note that a positive direct antiglobulin test was reported by Hyman and Southworth (1951) in four out of five patients It should be added however that Chaplin and Mollison (1953) found that the antiglobulin reaction was negative in the mildly anæmic cases they studied

The patient whose clinical history is described below was particularly interesting because anæmia developed acutely apparently as a consequence of liver necrosis In this instance there appeared to be some morphological evidence of toxic changes affecting the patient s erythrocytes

Case Report Acute Liver Necrosis Associated with Anæmia probably of Hæmolytic Type

Case 24 The patient (H D) was a married woman aged 31 years who was admitted to hospital in coma four days after being delivered of stillborn twins One month previously she had transient albuminuria but her blood pressure was normal and there did not seem to be any clear signs of pregnancy toxæmia On admission although unconscious she made some response to painful stimuli She was markedly jaundiced and the normal liver dullness appeared to be absent Her urine contained bile pigments A diagnosis of hepatic coma was made and the disease was attributed to acute viral hepatitis

She was treated initially by means of a protein free intra gastric drip containing dextrose Her coma diminished two days after admission and the jaundice disappeared after 14 days Her serum bilirubin reached a peak figure of 11 5 mg per 100 ml and her blood urea 90 mg per 100 ml after which the concentrations rapidly subsided Albuminuria and biliuria persisted for 12 days She ultimately made a complete recovery and a liver biopsy carried out two months after the start of her illness showed no signs of permanent liver damage

Laboratory Investigations On the day of admission (June 28th 19..) her erythrocyte count was 3 100 000 cells per c mm hæmoglobin 8 9 g

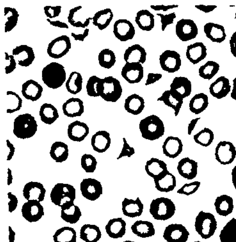


Fig. 87. Photomicrograph of a blood film of a patient suffering from acute liver necrosis and probable hæmolytic anæmia (Case [illegible]4) × [illegible]00

per 100 ml M C V [illegible] c μ and reticulocytes 4 [illegible]% The total leucocyte count was 38 000 cells per c mm with 64% neutrophils and 4% myelocytes Spectroscopic examination of her plasma for oxyhæmoglobin and methæmalbumin was negative The direct antiglobulin test was negative

Stained blood films showed marked anisocytosis and a moderate number of polychromatic macrocytes Some target cells could also be seen In addition small numbers of irregularly contracted corpuscles were present some being almost triangular in shape (Fig 87) About 9 [illegible]00 normoblasts per c mm were present An osmotic fragility test carried out on July 9th showed increased resistance as well as a small tail of fragile cells initial lysis 0 [illegible]% NaCl complete lysis 0 [illegible]0% NaCl M C F 0 37% NaCl

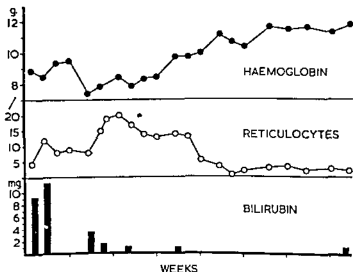


Fig 88 Hæmatological observations on a patient recovering from acute hepatic necrosis which was probably associated with hæmolytic anæmia (Case [illegible]4)

A bone marrow puncture on July 8th showed a markedly hypercellular marrow Erythropoiesis was normoblastic about [illegible]0% of all the nucleated cells being normoblasts in different stages of development Leucopoiesis was active but normal

The hæmatological changes during the patient s illness and recovery are shown in Fig 88 Her erythrocyte count and hæmoglobin concentration did not change substantially during the first 3 weeks of her illness despite a reticulocyte count of 8 to 21% (200 000 to 580 000 cells per c mm) during this time and it was thought that this indicated continuing hæmolysis of moderate degree Eventually her erythrocyte count and hæmoglobin gradually increased and the reticulocytosis

serum bilirubin and serum iron concentrations and in urobilinogen excretion the anæmia was largely hæmolytic in nature

Muirhead Jones Stirman and Lesch (1953) carried out similar studies in dogs but managed to keep the animal alive after the nephrectomies for up to 14 days or more by means of peritoneal dialysis A progressive anæmia was observed which could not be accounted for by blood loss Pigment studies again suggested that the anæmia was largely hæmolytic in nature although there was not always a close correlation between the pigment excretion and the degree of apparent erythrocyte destruction

There were important pathological changes in the spleen liver and lymph nodes hæmosiderosis was conspicuous and also erythrophagocytosis the latter being particularly noticeable within the lymph sinuses of the lymph nodes

Reticulocyte Counts in the Anæmia of Uræmia

The true incidence of reticulocytosis in severe anæmias associated with uræmia in man is unknown Judging from the literature (e g Lowinger 1938 Callen and Limarzi 1950) raised counts are rarely found—Callen and Limarzi reported for instance an average count of 0 7% in 44 patients with azotæmia However counts above the normal range have generally been observed in patients in whom an increased rate of hæmolysis has been demonstrated (Emerson 1948 Loge *et al* 1950 Chaplin and Mollison 1953)

The author's own observations suggest that in the terminal stages of acutely progressive uræmia markedly raised reticulocyte counts are not unusual It seems probable that in these patients some degree of increased hæmolysis will always be found if erythrocyte survival studies are carried out In Table 27 are illustrated the relevant hæmatological data obtained from five recent cases

Erythrocyte Morphology

The general opinion in the literature is that no noteworthy changes are to be found (Parsons and Ekola Strolberg 1933 Callen and Limarzi 1950) Except for Schwartz and Motto (1949) who described as burr cells certain deformed poikilocytes in cases of uræmia and carcinoma (see p 13) there seems to be no mention of possibly significant morphological changes In the author's series of severe anæmias in uræmic patients contracted and deformed erythrocytes have been frequently seen A characteristic type is an almost triangular cell but all stages in the process of contraction and distortion can as a rule be made out if films are carefully studied These changes can be seen in wet preparations of whole blood but it seems likely that the

diminished The contracted and distorted erythrocytes seen in blood films at the start of her illness persisted for about 2 weeks Moderate numbers of siderocytes were also present for about the same length of time Her leucocyte count varied between 18 000 and 38 000 per c mm for 9 days and myelocytes and normoblasts persisted in peripheral blood films for the first three weeks of her illness

Summary Acute hepatic necrosis (? due to viral hepatitis) with a leuco erythroblastic anæmia probably hæmolytic in type There was no evidence of auto immunization Recovery was slow but was eventually complete

HÆMOLYTIC ANÆMIA IN URÆMIA

Anæmia is a frequent accompaniment of renal disease in man Its exact cause is uncertain but it is clear that its severity is correlated with the severity of renal impairment The anæmia is unaccompanied by leucopenia or thrombocytopenia and bone marrow studies have shown that in the majority of cases the marrow is hyperplastic rather than hypoplastic with active leucocyte and thrombocyte production and with normal or increased erythropoietic activity (Callen and Limarzi 1950)

Until recently the general consensus of opinion seems to have been that a defect in erythropoiesis was the most likely cause of the anæmia (Parsons and Ekola Strolberg 1933 Nordenson 1938 Lowinger 1938 Callen and Limarzi 1950) However recent studies suggest that in some cases at least increased hæmolysis plays a significant role (Emerson 1948 Emerson and Burrows 1949 Loge Lange and Moore 1950 Hensley 1952 Chaplin and Mollison 1953)

Emerson (1948) described a patient who suffered from acute glomerulo nephritis During the acute phase of his disease transfused normal erythrocytes were eliminated at about three times the expected rate Emerson and Burrows (1949) described studies carried out on four patients suffering from chronic uræmia Evidence was obtained that hæmolysis was taking place at about one and a half to three times the normal rate

Chaplin and Mollison (1953) studied six patients suffering from rapidly progressive uræmia whose blood ureas ranged from 120 to 510 mg per 100 ml The survival of transfused normal erythrocytes was probably diminished in all of them in the three patients for whom sufficient data were available the mean cell life was calculated to be 16 32 and 59 days respectively On the other hand in three patients suffering from stationary chronic renal failure the transfused normal erythrocytes survived normally

Experimental studies have also shown that renal insufficiency lead to hæmolytic anæmia Muirhead Jones and Crollman (1952) carried out bilateral nephrectomy in rabbits The animals became anæmic during the three days that they survived and as judged by rises in

degree of contraction and distortion is exaggerated while the film dries While small numbers of distorted cells have been seen in films from most of the uræmic cases in some patients with severe progressive uræmia these cells have been present in large numbers (Fig 90) In these patients markedly raised reticulocyte counts in association with stationary or falling hæmoglobin concentrations suggested that blood destruction was taking place relatively rapidly In two patients small increases in osmotic fragility have been observed

Dacie and co-workers (1953 Case 11) reported that large numbers of triangular cells were present in the peripheral blood of a young girl thought to be suffering from a congenital hæmolytic anæmia Chronic nephritis giving rise to fatal uræmia was probably a factor in their development Splenectomy had been carried out and this too may have been a factor which led to the presence of such large numbers of these remarkable cells (Fig 5 p 14)

Pathogenesis of the Increased Hæmolysis in Uræmia

The cause of the increased blood destruction which may be a feature of some cases of uræmia is unknown Presumably a toxic factor of endogenous origin is responsible The exact cause of the uræmia seems immaterial but for blood destruction to be rapid rapidly progressive renal failure seems to be essential It is interesting to note that even where an increased rate of blood destruction has been established beyond question the serum bilirubin concentration may be within the normal range

The morphological abnormalities of the erythrocytes in the hæmolytic anæmia of uræmia are consistent with the hypothesis that the hæmolysis is brought about by a toxic factor The distortion is only obvious in mature non reticulated erythrocytes and develops presumably during the cells circulation the time relationship being analogous to that for the development of spherocytosis It is probably not a coincidence that similarly distorted corpuscles are seen in the peripheral blood of patients suffering from hæmolytic anæmia in carcinomatosis and in liver disease (Figs 4 and 87) where injurious metabolites also seem likely to be important factors in the pathogenesis of the erythrocyte destruction

There is little reason to suppose that auto immunization is an important factor in the causation of the hæmolytic anæmia of uræmia in most instances Serological tests have been negative in most of the patients in whom the tests have been carried out

Loge Lange and Moore (1950) and Chaplin and Mollison (19 3) reported negative direct antiglobulin tests in the cases of uræmia and hæmolytic anæmia they studied Hensley (1952) reported the

TABLE 27 *Hæmatological data in five patients with acutely progressive fatal uræmia*

Condition	Erythrocytes (millions per c mm)	Hæmoglobin (g per 100 ml)	M.C.V. c µ	Reticulocytes	Bilirubin mg per 100 ml	Direct antiglobulin test	Erythrocyte morphology
Acute cortical necrosis of kidney M 7 mths	3 7–3 8	5 8– 6 4	66	7–8		Negative	Many contracted cells mostly irregular in contour some triangular
Malignant hypertension (Case 25)	1 8–2 4	6 2– 8 4	102	15–22	0 5–0 7	Negative	Occasional contracted cells half moons and triangles (Fig 89)
Gouty nephrosis (Case 26)	2 5–2 9	8 1– 9 5	102	5–9	0 8	Positive	Many ovalocytes a few poikilocytes
Chronic nephritis (Case 27)	1 4–1 8	5 0– 6 0	109	29–41	0 9	Negative	Many contracted cells some rounded others irregular in shape or triangular Some very small schistocytes (Fig 90)
Malignant hypertension 1 97 *	3 0–3 5	10 3–11 9	95	7–13		Negative	Occasional contracted cells mostly of irregular contour

The survival of transfused normal blood was shown by Chaplin and Mollison (1953 Cases 9 and 10) to be impaired
denotes no observation

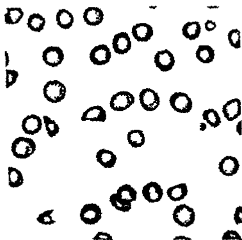


Fig 89 Photomicrograph of a blood film of a patient suffe ing from mal g ant hypertension and probable hæmolytic anæmia (Ca e 2) × 700

[To face p 368

antiglobulin test to be positive in one patient—this patient may have been suffering from acquired hæmolytic anæmia complicated by nephritis Of the author s cases in which hæmolysis was probably occurring the direct antiglobulin test was weakly positive in one patient only (Case 26)

Case Report Malignant Hypertension with Anuria and probable Hæmolytic Anæmia supervening on Pre eclamptic Toxæmia

Case 25 [1] The patient (M T) was a married woman aged 32 years Her illness started three years previously when she developed hyper tension during her first pregnancy Two months before her death she was admitted into hospital 26 weeks pregnant with marked albu minuria and œdema and a blood pressure of 180/110 Cæsarean section was performed but thereafter her condition steadily deteriorated A fortnight before her death she became anuric and her blood urea rose terminally to more than 400 mg per 100 ml

Laboratory Investigations Repeated observations were made during the last three weeks of her life during which time the blood picture remained comparatively unchanged The erythrocyte count varied between 1 700 000 and 2 400 000 cells per c mm and the hæmoglobin between 6 2 and 7 5 g per 100 ml the M C V averaged 104 c μ The reticulocyte count ranged between 15 and 23% the average of eight observations being 19% The total leucocyte count varied between 7 000 and 18 000 cells per c mm with an average of 85% neutrophils A single platelet count of 80 000 was recorded The serum bilirubin concentration varied between 0 5 and 0 7 mg per 100 ml Schumm s test was found to be weakly positive on one occasion The direct anti globulin test was negative The erythrocyte osmotic fragility was normal initial lysis 0 5% NaCl M C F 0 43% complete lysis 0 30% NaCl

Stained blood films showed a moderate degree of anisocytosis and polychromasia occasional distorted and contracted corpuscles and a few triangular forms were present (Fig 89)

A *postmortem* examination confirmed the clinical diagnosis of malig nant hypertension Sections of the spleen showed a congested pulp with much iron containing pigment in macrophages Erythrophago cytosis was however difficult to make out The Kupffer cells of the liver also contained iron

Case Report Uræmia due to ? Gouty Nephrosis and probable Hæmolytic Anæmia

Case 26 The patient (H D) was a man aged 65 years He was admitted to hospital complaining of general malaise and vomiting having been well until about one month previously He appeared to have had no significant illnesses in the past but there was a clear history of occasional attacks of gout extending back over the previous ten years

On admission he was found to be drowsy and disorientated and to have urinary retention A clinical diagnosis of uræmia was made His

[1] A detailed account of this case is given by Counihan and Donrach (*J Obstet Gynæc Brit Emp* (1954) In press)

blood pressure was 195/100 and the blood urea 100 mg per 100 ml The urine contained a moderate amount of albumin

His condition steadily deteriorated and he died 16 days after admission the blood urea at one time exceeding 300 mg per 100 ml

Laboratory Investigations The erythrocyte count ranged from 2,500 000 to 2 900 000 cells per c mm and the hæmoglobin from 8 1 to 9 5 g per 100 ml the M C V averaged 102 c μ The reticulocyte count ranged between 5 and 9% and the total leucocyte count between 4 000 and 2? 000 cells per c mm with 90% neutrophils A single platelet count of ?00 000 per c mm was recorded

The direct antiglobulin test was positive using strong concentrations of antiglobulin serum the reaction was not of the γ-globulin type The cold agglutinin titre was 256 at 2 C but less than 4 at 2? C No abnormal warm antibodies could be demonstrated The serum protein concentration was ? ? g per 100 ml with 3 ? g albumin and 4 0 g globulin Erythrocyte osmotic fragility was normal except for a small tail of fragile cells initial lysis 0 55% NaCl M C F 0 41% NaCl complete lysis 0 20% NaCl The serum bilirubin concentration averaged 0 8 mg per 100 ml

Stained films of his peripheral blood showed a moderate degree of anisocytosis and polychromasia a definite tendency to ovalocytosis and slight spherocytosis and a very few irregularly contracted corpuscles Bone marrow puncture revealed marrow of normal cellularity with a myeloid-erythroid ratio of 10 1 Erythropoiesis was normoblastic

A *postmortem* examination confirmed the tentative clinical diagnosis of gouty nephrosis with terminal heart failure and uræmic œdema of the lungs Sections of the spleen showed hyperplasia of reticulum cells some erythrophagocytosis and a great deal of hæmosiderin mostly in macrophages The bone marrow was moderately hyperplastic and erythropoiesis was conspicuous

Summary A case of uræmia due to gouty nephrosis with probable hæmolytic anæmia The direct antiglobulin reaction was weakly positive

Case Report Hæmolytic Anæmia Associated with Malignant Hypertension

Case 27 The patient (J G) was a man aged 35 years He was admitted to hospital giving a history that for the last three weeks he had been troubled with weakness anorexia abdominal pain bleeding from the nose cough and blurred vision Previous to this he had been well and there was no history of renal disease

He was a pale breathless man who appeared to be severely uræmic His blood pressure was 218/132 and he had advanced hypertensive retinitis His general condition gradually deteriorated and he died eight days after admission

Laboratory Investigations The erythrocyte count was almost constant at about 1 ?00 000 cells per c mm and the hæmoglobin ranged between 5 0 to 5 5 g per 100 ml The reticulocyte count varied between 19 and 41% and the total leucocyte count between 9 000 and 12 000 cells per c mm with 68 to 91% neutrophils There were 105 000 platelets per c mm

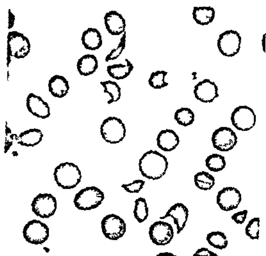


Fig 90 Photomicrograph of a blood film of a patient suffering from malignant hypertension and hæmolytic anæmia (Case 27) × 700

logical disturbances often leading to coma The first report of this syndrome seems to be that of Moschcowitz (1925) who described the clinical history of a girl aged sixteen who died of an acute febrile pleiochromic anæmia Microscopical examination of her tissues at postmortem revealed numerous hyaline thrombotic masses occluding the small vessels in many of the internal organs Four apparently similar cases were described by Baehr Klemperer and Schifrin (1936) who suggested that the thrombi were formed by platelets By 1947 Singer Bornstein and Wile were able to trace records of twelve patients including one of their own and suggested the title thrombotic thrombocytopenic purpura as an appropriate one for the syndrome Since then further cases have been reported and the syndrome has become well recognized Recent reviews include those of Rackow Steingold and Wood (1952) and Symmers (1952) who referred to 83 cases The hæmatological features of the syndrome will be briefly reviewed

Hæmatological Findings A characteristic feature in almost all the patients with the syndrome has been the rapid development of a severe hæmolytic anæmia The erythrocytes have usually been stated to be normocytic and spherocytosis has sometimes been noted The reticulocyte count has been raised almost invariably and counts as high as 51% have been recorded (Muirhead Crass and Hill 1948) The erythrocyte osmotic fragility has been reported to be normal or increased The leucocyte count is usually raised and counts as high as 50 000 cells per c mm the majority being neutrophils have been reported Small numbers of myelocytes and normoblasts are usually present The platelet count is characteristically reduced

Serological studies have seldom been attempted and in the few cases in which the antiglobulin test was carried out the direct reaction has been reported as negative (Singer Motulsky and Shanberge 1950 Meacham *et al* 1951) However in the personally observed case reported below the test was weakly but definitely positive

Pathogenesis Little is known of the cause or pathogenesis of thrombotic thrombocytopenic purpura It is possible that it is a type of collagen disease and that the underlying basis is one of hypersensitivity The exact mechanism of the hæmolysis is unknown and auto antibodies of the type met with in typical idiopathic acquired hæmolytic anæmia do not seem to be formed The disease can occur in a fulminating form even after splenectomy as the following case report demonstrates

The direct antiglobulin test was negative and the serum bilirubin concentration 0 9 mg per 100 ml His blood urea on admission was 373 mg per 100 ml and this rose terminally to 510 mg per 100 ml

The erythrocyte osmotic fragility was increased there being a small number of fragile cells lysis commenced in 0 6% NaCl the M C F being 0 40% NaCl

Stained blood films were remarkable for the large numbers of distorted and contracted cells and cell fragments that were present (Fig 90) There was also conspicuous polychromasia Five days before he died he was transfused with 250 ml of normal group O packed cells The survival of this blood was shown by the Ashby method to be definitely impaired (Chaplin and Mollison 1953 Case 9)

Postmortem Examination The main features were as follows Œdema of the lungs left ventricular hypertrophy and kidney changes suggestive of malignant hypertension The cavity of the upper two thirds of the femur was filled with active marrow Sections confirmed the diagnosis of malignant hypertension The bone marrow in the sternum and femur was markedly hyperplastic and predominantly erythropoietic and there was a good deal of erythrophagocytosis

Summary A case of rapidly progressive fatal uræmia due to malignant hypertension Severe anæmia of hæmolytic type with marked erythrocyte contraction and distortion and a high reticulocytosis

Intravascular Hæmolysis in Eclampsia

It has long been known that hæmoglobinæmia and hæmoglobinuria may be found in the acute phase of eclampsia Young (1942) referred to a number of records in the German literature of the previous 60 years Recently the subject has once more received attention A single case report was published by Kistner and Assali (1950) and a series of patients investigated by Pritchard Weisman Ratnoff and Vosburgh (1953 1954) The cause of the acute hæmolytic episodes is unknown

Pritchard and co workers (1953) studied eleven eclamptic women and found some evidence of increased hæmolysis and/or hæmorrhagic phenomena in all of them Three patients suffered from transient gross hæmoglobinæmia and hæmoglobinuria and in six others a hæmolytic process was strongly suspected In two patients erythrophagocytosis was observed in buffy coat preparations of peripheral blood and the erythrocyte osmotic fragility was slightly increased In two patients too the direct antiglobulin test was found to be transiently positive No detailed studies on erythrocyte morphology were carried out

THROMBOTIC THROMBOCYTOPENIC PURPURA

Within recent years a fatal syndrome has become widely recognized consisting of hæmolytic anæmia and thrombocytopenic purpura associated with azotæmia and with fluctuating neuro

logical disturbances often leading to coma The first report of this syndrome seems to be that of Moschcowitz (1925) who described the clinical history of a girl aged sixteen who died of an acute febrile pleiochromic anemia Microscopical examination of her tissues at postmortem revealed numerous hyaline thrombotic masses occluding the small vessels in many of the internal organs Four apparently similar cases were described by Baehr Klemperer and Schifrin (1936) who suggested that the thrombi were formed by platelets By 1947 Singer Bornstein and Wile were able to trace records of twelve patients including one of their own and suggested the title thrombotic thrombocytopenic purpura as an appropriate one for the syndrome Since then further cases have been reported and the syndrome has become well recognized Recent reviews include those of Rackow Steingold and Wood (1952) and Symmers (1952) who referred to 33 cases The hæmatological features of the syndrome will be briefly reviewed

Hæmatological Findings A characteristic feature in almost all the patients with the syndrome has been the rapid development of a severe hæmolytic anæmia The erythrocytes have usually been stated to be normocytic and spherocytosis has sometimes been noted The reticulocyte count has been raised almost invariably and counts as high as 51 % have been recorded (Muirhead Crass and Hill 1948) The erythrocyte osmotic fragility has been reported to be normal or increased The leucocyte count is usually raised and counts as high as 50 000 cells per c mm the majority being neutrophils have been reported Small numbers of myelocytes and normoblasts are usually present The platelet count is characteristically reduced

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Pathogenesis Little is known of the cause or pathogenesis of thrombotic thrombocytopenic purpura It is possible that it is a type of collagen disease and that the underlying basis is one of hypersensitivity The exact mechanism of the hæmolysis is unknown and auto antibodies of the type met with in typical idiopathic acquired hæmolytic anæmia do not seem to be formed The disease can occur in a fulminating form even after splenectomy as the following case report demonstrates

Case Report Fatal Thrombotic Thrombocytopenic Purpura

Case 28 The patient (M S) was a widow aged 56 years who had previously undergone hysterectomy two operations for intestinal obstruction and ultimately gastrectomy (and splenectomy) in November 1950 Two weeks before her final admission into hospital in March 1951 she started to vomit occasionally On the day before admission she was noticed to be drowsy

Physical Examination She was a wasted woman in a semi comatose condition There were multiple abdominal scars otherwise no abnormal physical signs were noted except that the reflexes were brisker on the left than on the right side The day after admission slight jaundice was noticed and some purpura of the skin Her coma increased in depth and she died on the following day Her urine was dark brown in colour it contained much albumin some granular casts a few leucocytes and an occasional erythrocyte Spectroscopic examination revealed large amounts of methæmoglobin A tentative diagnosis of thrombotic thrombocytopenic purpura was made

Laboratory Findings The hæmoglobin concentration was 14 2 g per 100 ml reticulocyte count 1 2% leucocyte count 18 000 cells per c mm 89% of which were neutrophils and the platelet count 10 000 per c mm The bleeding time was in excess of 18 minutes and the coagulation time 6¾ minutes

Stained blood films showed a moderate anisocytosis and occasional Howell Jolly bodies and Pappenheimer bodies Platelets were few some being unusually large misshapen and densely staining The plasma had a brownish tinge and was found to contain small amount of methæmalbumin

The direct antiglobulin test was weakly positive but no abnormal antibodies could be detected in the patient s serum using trypsinized and P N H erythrocytes The cold agglutinin titre was 8 at 2° C and the serum complement concentration normal A biopsy of the sternal bone marrow revealed moderately cellular marrow containing plenty of megakaryocytes but with little evidence of actual platelet formation

Postmortem Examination The most important macroscopic findings were abdominal adhesions slightly swollen kidneys with small hæmorrhages on their surfaces and a slightly swollen brain Microscopically[1] the only important lesions were widespread small thrombi in capillaries arterioles and some venules The thrombi were present in very large numbers in the kidneys they were fairly numerous in the brain and present in smaller numbers in the thyroid liver lungs pancreas and subcutaneous fat but were not found in the myocardium adrenals or bone marrow In addition to the capillary thrombi sections of the kidneys showed many pigment containing casts in the renal tubules

Summary A fatal illness of short duration starting with vomiting and leading to death in coma There was marked thrombocytopenia and evidence suggestive of an episode of intravascular hæmolysis Postmortem examination revealed widespread capillary thromboses

[1] Histological preparations from this case were shown by B Lennox and J V Dacie at the International Congress of Clinical Pathology in London in 1951

HÆMOLYTIC ANÆMIA IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS

Anæmia is almost invariably present in acute disseminated lupus erythematosus. Michael, Vural, Bassen and Schaefer (1951) who reviewed the hæmatological data obtained from 111 patients recorded hæmoglobin levels varying from 2.5 g. to 15 g. per 100 ml.; 10% of the patients had hæmoglobin concentrations of less than 1[illegible] g. per 100 ml. at one time or another. The anæmia is usually normocytic and normochromic and the reticulocyte count is often slightly raised. As is well known, leukopenia and thrombocytopenia often occur.

Occasionally severe and obviously hæmolytic anæmia develops. Michael and co-workers (1951) reported three such cases, anæmia being the presenting sign in one patient, while Pisciotta and co-workers (1951) observed acute hæmolytic anæmia in one out of seven patients studied. Dubois (1952) described three patients in each of whom an acute hæmolytic anæmia was the presenting *sign of their illness. He also mentioned that six out of nine other* patients suffering from disseminated lupus showed some signs of increased hæmolysis. A further example of severe hæmolytic anæmia was described by Baikie (1953).

The true incidence of accelerated hæmolysis in disseminated lupus erythematosus is not yet known. It seems likely, however, that minor degrees of increased hæmolysis would be frequently found if careful erythrocyte survival studies were carried out.

Serological Findings. The direct antiglobulin test has been found to be positive in nearly all the reported cases with overt hæmolytic anæmia (e.g. Pisciotta *et al.*, 1951; Dubois, 1952; Baikie, 1953). Baikie also demonstrated an auto-agglutinin in the patient's serum active at 37° C. Positive direct antiglobulin tests have also been recorded in patients who have not been suffering from clinically obvious hæmolytic anæmia (Evans *et al.*, 1951; Baikie, 1953).

Antiglobulin tests have been carried out in the author's laboratory on the erythrocytes of nine patients: the reaction was strongly positive in one patient suffering from overt hæmolytic anæmia, and weakly positive in five out of eight patients without obvious hæmolytic anæmia. In each case the reaction appeared to be of the "cold antibody" type, i.e. it was not inhibited by small concentrations of γ-globulin.

As referred to on p. 297, multiple immune antibodies are

frequently formed by patients who are repeatedly transfused (see also Kuhns and Bauerlein 1953)

Hæmolytic Anæmia in Association with Periarteritis Nodosa

A small number of cases have been reported in which hæmolytic anæmia has been associated with or followed by periarteritis nodosa

Dameshek and Rosenthal (1951) *referred to four patients whose* hæmolytic disease was not benefited by splenectomy and in whom histological evidence of arteritis was found at postmortem They also described two further possible examples the arteritis being demonstrated histologically by biopsy in one case Both patients responded extremely well to A C T H therapy in one instance after splenectomy had failed to bring relief The direct antiglobulin test was positive in both patients and warm auto antibodies were demonstrated in their sera

Hæmolytic Anæmia Associated with Boeck s Sarcoid

Several instances of hæmolytic anæmia have been reported in patients who have subsequently been shown to suffer from Boeck s sarcoid The exact relationship between the two disease *processes is obscure However it is perhaps noteworthy* that as in idiopathic acquired hæmolytic anæmia hyperglobulinæmia is commonly found in cases of sarcoidosis (McCort Wood Hamilton and Ehrlich 1947 Ricker and Clark 1949)

Crane and Zetlin (1945) described a patient aged 46 who underwent splenectomy for acquired hæmolytic anæmia but relapsed two months later There was marked spherocytosis high reticulocytosis and a plasma globulin concentration of 4 g per 100 ml The patient died and histological examination showed the characteristic signs of sarcoid in lymph nodes and bone marrow

Stats Rosenthal and Wasserman (1947) reported the history of a child who developed a severe hæmolytic anæmia when six months of age He recovered from this but relapsed when aged seven years febrile episodes then being accompanied by signs of hæmolysis A year later with the hæmolytic anæmia persisting iridocyclitis developed and *lymph node biopsy revealed sarcoidosis*

McCort and co workers (1947) referred to a man aged 58 who was known to have enlarged mediastinal glands the histological picture of which was typical of sarcoid Two years later a hæmolytic anæmia developed which was not improved by splenectomy Radiographs of his chest showed that the mediastinal nodes had increased in size

Bruschi and Howe (1950) reviewed the blood changes which had been recorded in sarcoidosis and described a further case of severe hæmolytic anæmia Splenectomy was carried out in this patient with temporary improvement in the blood picture Sarcoid tissue was demonstrated histologically in the spleen in a splenic lymph node and in the liver

CHRONIC IDIOPATHIC ACQUIRED HÆMOLYTIC ANÆMIA HYPERSPLENISM

The great majority of cases of chronic acquired hæmolytic anæmia are undoubtedly caused by anti erythrocyte auto anti bodies Occasionally however patients in whom no evidence of auto immunization can be detected present with all the signs of a chronic hæmolytic anæmia Some of these patients will be found to be suffering from an underlying disease such as lymphadenoma others may in reality be suffering from paroxysmal nocturnal hæmoglobinuria But leaving aside these possibilities there remain a few patients whose hæmolytic anæmia cannot be explained some of them if they have splenomegaly may be suffering from primary hypersplenism It seems likely however that the number of cases which can be adequately explained on the theory that the spleen is the primary and sole cause of the hæmolysis is very small indeed Most of the patients in whom a diagnosis of hypersplenism has been made seem likely in retrospect to have been suffering from acquired hæmolytic anæmia of the auto antibody type

A possible example of hypersplenic hæmolytic anæmia has been recently recorded by Dausset Paraf and Caroli (195_) The patient was severely anæmic and the leucocyte and platelet counts were also moderately reduced All tests for abnormal antibodies were negative Splenectomy resulted in a prompt cessation of hæmolysis and a return to normal of the leucocyte and platelet counts

The author has not studied any patient whose hæmolytic anæmia could be ascribed to primary hypersplenism with any confidence Two patients however have been investigated whose chronic hæmolytic anæmia has been completely unclassifiable The first patient (Case 29) eventually recovered spontaneously from her anæmia her spleen was never palpable and she can hardly be regarded as a case of hypersplenism The second patient (Case 30) had an equally unexplained hæmolytic anæmia Her spleen was however palpable and the excessive hæmolysis subsided after splenectomy It is possible therefore that she was in fact suffering from hypersplenism

Case Report Chronic Acquired Hæmolytic Anæmia of Unknown Ætiology and Pathogenesis

Case 29 The patient (M O) was a married woman aged 70 years She was admitted to hospital in January 1950 complaining that she had felt breathless and weak for the past month Previously to this her health had been good

Physical Examination She was a well nourished elderly woman who was pale and slightly jaundiced There were no significantly abnormal physical signs her liver and spleen were not palpable nor were there any enlarged lymph nodes Her urine contained an excess of urobilin

Laboratory Investigations Whilst under observation for two weeks her erythrocyte count fell from 2 200 000 to 1 700 000 cells per c mm and her hæmoglobin concentration from 8 6 to 7 0 g per 100 ml The M C V averaged 132 c μ The reticulocyte count varied between 23 and 31% and the serum bilirubin concentration between 0 7 and 1 2 mg per 100 ml The total leucocyte count varied between 7 000 and 11 000 cells per c mm with 67% neutrophils and there were 460 000 platelets per c mm Stained films showed many macrocytes a few pear shaped poikilocytes and considerable polychromasia No spherocytes were seen

The osmotic fragility was not increased initial lysis 0 45% NaCl M C F 0 40% NaCl The rate of autohæmolysis of defibrinated blood incubated at 37 C for 48 hours was slightly less than twice that of a normal control

The direct antiglobulin test was negative and no abnormal warm antibodies could be detected in her serum the cold agglutinin titre was < 4 at 2 C The Wassermann and Kahn tests were negative Her blood group was A MN Rh positive

She was transfused with two pints of group A N Rh positive blood The survival of the normal erythrocytes was followed by the Ashby method using an anti M serum The transfused cells were completely eliminated in 17 days the mean cell life being about 4 days

Subsequent Course The patient has been seen at intervals for over four years since her admission into hospital By March 1950 her blood count had become stabilized at about 2 500 000 erythrocytes per c mm with 10 0 g hæmoglobin per 100 ml and 20% reticulocytes Thereafter the blood count gradually improved One year later the erythrocyte count had risen to 3 200 000 cells per c mm with 11 4 g hæmoglobin per 100 ml and 8% reticulocytes When last seen in March 1954 her blood count was almost normal with 4 100 000 erythrocytes per c mm 13 2 g hæmoglobin per 100 ml and 2 4% reticulocytes

Summary A case of acquired hæmolytic anæmia of unknown type with no evidence of auto immunization Normal leucocyte and platelet counts Slow spontaneous recovery

Case Report Chronic Idiopathic Acquired Hæmolytic Anæmia Associated with Panhypopituitarism (? Simmonds's Disease)

Case 30 The patient (N C) was a married woman aged 37 years She was admitted to hospital in October 1949 complaining of amenorrhœa She had had a severe post partum hæmorrhage in 1938 after the birth of her sixth child and menstruation had been scanty ever since

Physical Examination On admission into hospital she was found to be pale and overweight and to present a rather myxœdematous appearance her skin was dry and she had lost some of her axillary and pubic hair The spleen was just palpable

Laboratory Investigations There were 3 000 000 erythrocytes per c mm and 10 2 to 11 7 g of hæmoglobin per 100 ml The reticulocyte

count varied between 8 and 2⁻ and the total leucocyte count from _ ⁻00 to 4 000 per c mm *of which between 40 and 63 ₀ were neutrophils* The platelet count varied between 145 000 and 190 000 per c mm Stained peripheral blood films showed that the majority of the erythrocytes were round or oval normocytes but some polychromatic microcytes and a few microcytes were also present

The erythrocyte osmotic fragility was normal initial lysis 0 50 NaCl M C F 0 4 °₀ NaCl The rate of autohæmolysis was between two and three times that of a normal control

The plasma bilirubin concentration ranged between 0 8 and 2 2 mg per 100 ml Schumm s test was negative The direct antiglobulin test was negative and tests for abnormal warm antibodies in the serum were al o negative The cold agglutinin titre was 4 at 2 C The acidified erum test wa negative

She was transfused both before and immediately after splenectomy The survival of the normal erythrocytes was definitely impaired before splenectomy ₅0° of the transfu ed cells had been destroyed seventeen days after the transfusion and less than 10°₀ remained after 37 days After splenectomy the erythrocytes survived normally (Mollison 19₅1)

Splenectomy Her spleen was removed in March 19₅0 it weighed 4₅0 g Sections showed that the general pattern was preserved the littoral cell of the sinu es were prominent and the pulp contained a moderate amount of blood Erythrophagocytosis could be seen but not in excessive amounts

Subsequent Progress The initial effect of splenectomy was good the erythrocyte count and hæmoglobin concentration reached normal levels and remained so for at least six months the reticulocyte count varied between 0 5 and 3°₀ The leucocyte count and platelet counts also became normal

When seen three year later she showed signs of more marked hypopituitarism The erythrocyte count had fallen to 3 100 000 per c mm the hæmoglobin concentration to 10 2 g per 100 ml and there were 3 to 4°₀ reticulocytes The leucocyte count varied between 7 000 and 11 000 cells per c mm with _ to 45 % neutrophils

Summary A case of acquired hæmolytic anæmia of unknown type and causation associated with mild panhypopituitari m Splenectomy resulted in a good remi sion Three years later there were sign of hæmatological and endocrinological relapse

Hæmolytic Anæmia in Pregnancy

Many different types of chronic hæmolytic anæmia may be met with in pregnant women and acute sometimes transient episodes occurring usually tov ard the end of pregnancy or after parturition are rare but not unknown (Lescher 194) However a clear cut causal relation hip between the pregnancy and the hæmolysis has seldom been established The case reports of Bromberg Toaff and Ehrenfeld (1948) and Zachariae (19 3) do nevertheless suggest that pregnancy may be a cau e of acute exacerbations in certain types of chronic idiopathic acquired hæmolytic anæmia for both the patients described in the above mentioned reports developed serious hæmolytic epi o les in successive pregnancies

It should be added that minor increa es in osmotic fragility may be found in both anæmic and non anæmic normal pregnant women

Physical Examination She was a well nourished elderly woman who was pale and slightly jaundiced There were no significantly abnormal physical signs her liver and spleen were not palpable nor were there any enlarged lymph nodes Her urine contained an excess of urobilin

Laboratory Investigations Whilst under observation for two weeks her erythrocyte count fell from 2 200 000 to 1 700 000 cells per c mm and her hæmoglobin concentration from 8 6 to 7 0 g per 100 ml The M C V averaged 132 c μ The reticulocyte count varied between 23 and 31% and the serum bilirubin concentration between 0 7 and 1 2 mg per 100 ml The total leucocyte count varied between 7 000 and 11 000 cells per c mm with 67% neutrophils and there were 460 000 platelets per c mm Stained films showed many macrocytes a few pear shaped poikilocytes and considerable polychromasia No spherocytes were seen

The osmotic fragility was not increased initial lysis 0 45% NaCl M C F 0 40% NaCl The rate of autohæmolysis of defibrinated blood incubated at 37 C for 48 hours was slightly less than twice that of a normal control

The direct antiglobulin test was negative and no abnormal warm antibodies could be detected in her serum the cold agglutinin titre was < 4 at 2 C The Wassermann and Kahn tests were negative Her blood group was A MN Rh positive

She was transfused with two pints of group A N Rh positive blood The survival of the normal erythrocytes was followed by the Ashby method using an anti M serum The transfused cells were completely eliminated in 17 days the mean cell life being about 4 days

Subsequent Course The patient has been seen at intervals for over four years since her admission into hospital By March 1950 her blood count had become stabilized at about 2 500 000 erythrocytes per c mm with 10 0 g hæmoglobin per 100 ml and 20% reticulocytes Thereafter the blood count gradually improved One year later the erythrocyte count had risen to 3 200 000 cells per c mm with 11 4 g hæmoglobin per 100 ml and 8% reticulocytes When last seen in March 1954 her blood count was almost normal with 4 100 000 erythrocytes per c mm 13 2 g hæmoglobin per 100 ml and 2 4% reticulocytes

Summary A case of acquired hæmolytic anæmia of unknown type with no evidence of auto immunization Normal leucocyte and platelet counts Slow spontaneous recovery

Case Report Chronic Idiopathic Acquired Hæmolytic Anæmia Associated with Panhypopituitarism (? Simmonds's Disease)

Case 30 The patient (N C) was a married woman aged 37 years She was admitted to hospital in October 1949 complaining of amenorrhœa She had had a severe post partum hæmorrhage in 1938 after the birth of her sixth child and menstruation had been scanty ever since

Physical Examination On admission into hospital she was found to be pale and overweight and to present a rather myxœdematous appearance her skin was dry and she had lost some of her axillary and pubic hair The spleen was just palpable

Laboratory Investigations There were 3 000 000 erythrocytes per c mm and 10 2 to 11 7 g of hæmoglobin per 100 ml The reticulocyte

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(Elliott 1944) The significance of this finding is uncertain it should however engender caution in diagnosing an anæmia of pregnancy as hæmolytic solely on the results of the osmotic fragility test

MARCH HÆMOGLOBINURIA

March or *exercise* hæmoglobinuria was first described in Germany by *Fleischer* in *1881* The condition is characterized by the sudden appearance of hæmoglobin in the urine following exertion in an otherwise healthy subject The hæmoglobinuria is not related to sleep or cold or as far as is known to disease of any sort and the loss of hæmoglobin and the frequency of the attacks are not as a rule sufficient to cause an appreciable degree of chronic anæmia March hæmoglobinuria has quite a large literature and was the subject of a detailed review by Gilligan and Blumgart *(1941)* *It is primarily a disorder of males although in at least* two instances women have been affected (Gilligan and Altschule 1950) As a rule spontaneous recovery takes place within a few months or a year or so

Pathogenesis The cause and mechanism of march hæmoglobinuria is obscure It is known that the hæmoglobinuria is secondary to hæmoglobinæmia and that the hæmolysis giving rise to the hæmoglobinæmia occurs only at the time of the exertion Gilligan and Blumgart (1941) concluded that an appropriate (lordotic) posture was necessary to bring on an attack of hæmoglobinuria in a sensitive subject in addition to exercise Gilligan and Blumgart also pointed out that physiological hæmoglobinæmia and hæmoglobinuria occurred not uncommonly in normal people after extremely severe and prolonged exertion they postulated that in people subject to march hæmoglobinuria hæmolysis developed following lesser degrees of exertion as the result of some undefined mechanical or postural abnormality It may be added that Gilligan and Blumgart (1941) and Hobbs (1944) found that the mechanical fragility *in vitro* of the erythrocytes of affected men was normal No real advances in the understanding of this strange disorder seem to have been made in recent years

Pare and Sandler (1954) have recently reported that the urine of sufferers from march hæmoglobinuria contains abnormal amounts of amino acids such as cystine and β amino isobutyric acid This finding however does not seem to throw any light upon the mechanism of hæmolysis

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(1933). Following these publications both drugs were for a time widely used in the therapy of polycythæmia. The treatment was, however, potentially dangerous for it was not easy to regulate the dose so as to avoid hæmolytic episodes due to cumulative effects (McCance and Widdowson, 1937).

Phenylhydrazine and acetylphenylhydrazine affect erythrocytes in several ways. In man even relatively small doses cause visible damage to the erythrocytes: some of the cells undergo contraction and may appear as if pieces have been eaten out of their periphery (Fig. p. 12); others show patchy deficiencies in hæmoglobin. Regenerating cells are also affected, as is shown by the coarse punctate basophilia and siderotic granules in stained films. In addition, the hæmoglobin itself is altered so that Heinz bodies are formed (Webster, 1949; Beaven and White, 1954; see also p. 14).

If present in large numbers Heinz bodies give the blood a curious brownish colouration rather like that due to large amounts of sulphæmoglobin or methæmoglobin. Heinz bodies, however, consist of denatured globin, and neither sulphæmoglobin nor methæmoglobin is formed as the result of the action of phenylhydrazine or acetylphenylhydrazine. When isolated, Heinz bodies are a greenish brown in colour; they are insoluble in water and this causes a persistent turbidity when blood is laked in distilled water or hypotonic saline. The presence of intracorpuscular Heinz bodies leads to an increase in resistance to lysis by hypotonic saline and, in association with the increased fragility of corpuscles which have undergone irreversible contraction, to an increased span of resistance in osmotic fragility tests.

In severe overdosage with phenylhydrazine or acetylphenylhydrazine the patient becomes cyanosed from Heinz body formation resulting in a serious impairment of the oxygen-carrying capacity of the blood. In addition, he will be pale and jaundiced and there may be hæmoglobinuria and methæmoglobinuria. The blood has a chocolate-brown tinge, and the plasma contains hæmoglobin and methæmalbumin as well as an increased content of bilirubin. Autohæmolysis *in vitro* is unusually rapid.

The following case history illustrates the results of serious self-inflicted poisoning with acetylphenylhydrazine. The patient had actually undergone splenectomy before the source of the hæmolytic anæmia was discovered. (*Spontaneous Heinz body anæmia* is discussed on p. 399.)

Case Report. Acetylphenylhydrazine Hæmolytic Anæmia. Splenectomy.

Case 31. The patient was an unmarried woman aged 27 years. Between 1941 and 1945 she suffered from occasional fainting attacks and a diagnosis of "cryptogenic epilepsy" was made. In January and in

CHAPTER 15

HÆMOLYTIC ANÆMIAS DUE TO DRUGS, CHEMICALS AND INFECTIONS

Hæmolytic Anæmia due to Drugs or Chemicals

MANY drugs and chemicals are known to cause hæmolysis when administered to man or animals Some do this regularly if given in sufficient dosage although there are certain differences in sensitivity amongst individuals or species other drugs or chemicals cause hæmolytic anæmia much more capriciously and affect only a small proportion of patients or animals In the latter group the onset of hæmolysis appears to be due to an unusual susceptibility and is not closely connected with dosage

The exact way in which drugs or chemicals cause hæmolysis *in vivo* is incompletely understood in most instances There are at least three possible mechanisms the hæmolysis may be brought about (*a*) by a direct action on the circulating and developing erythrocytes (*b*) through the intermediary of abnormal metabolic products—in which case only a small percentage of patients or animals may be affected and (*c*) by the chemical acting as a pro antigen which in combination with the patient s erythrocytes causes auto immunization

In this chapter a brief account will first be given of the chemically induced hæmolytic anæmias of more or less regular occurrence this will be followed by a description of those in which an unusual degree of susceptibility appears to be an important factor in the pathogenesis of the anæmia (*chemical hæmolytic* anæmias of the hypersensitivity type)

Phenylhydrazine and Acetylphenylhydrazine

Phenylhydrazine and its acetyl derivative (pyrodin) have been used repeatedly in experimental studies on hæmolytic anæmia in animals (Cruz 1941) In man phenylhydrazine was apparently first used in polycythæmia vera as a means of reducing the erythrocyte count by Eppinger and Kloss (1918) the acetyl derivative was later employed for the same purpose by Bassett Killip and McCann (1931) and by Stone Harris and Bodansky

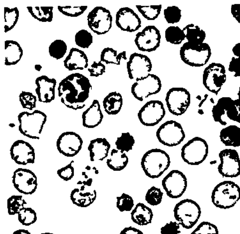


Fig. 9. Photomicrograph of a blood film of a patient suffering from acetylphenylhydrazine poisoning (after splenectomy) (Case 31) × 700

December 1948 whilst working as a pharmacist she was treated for anxiety neurosis In January 1949 she developed a severe hæmolytic anæmia which was controlled only by successive blood transfusions The cause of the anæmia was obscure but as there seemed to be no signs of any improvement splenectomy was carried out on April 6th 1949 The immediate effects of the operation were good the hæmoglobin concentration was sustained and the reticulocyte count fell to normal levels

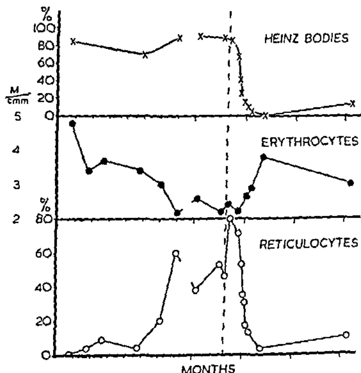


FIG 91 Hæmatological observations on a patient suffering from acetylphenylhydrazine poisoning (Case 31) The vertical interrupted line indicates the point at which the patient stopped taking the drug

The patient was seen in London on May 26th 1949 She appeared to be perfectly well and was not anæmic There were 4 800 000 erythrocytes per c mm a packed cell volume of 41% and 0 5% reticulocytes A cresyl blue stained blood film however showed that over 80% of her corpuscles contained large Heinz bodies (Fig 9 p 16) Romanowsky stained films were normal in appearance except for occasional Howell Jolly bodies and numerous Pappenheimer bodies

The patient was seen at about monthly intervals until September 1949 She had kept well but had become slightly anæmic the erythrocyte count falling to 3,500 000 cells per c mm Heinz bodies

were still present in very large numbers and the reticulocyte count was slightly increased. Occasional cells showing diffuse punctate basophilia could be seen in Romanowsky stained films and the Heinz bodies could be identified in some cells as rounded areas surrounded by a slightly deeper coloured rim.

On November 27th 1949 there were definite signs of relapse (Fig. 91). Stained blood films showed many irregularly crenated and distorted erythrocytes and marked punctate basophilia; pale lilac ring-like bodies of irregular contour corresponding in size to Heinz bodies could be seen in many of the cells, particularly in the most contracted cells (see p. 401). The reticulocyte count had risen to 20%. Thereafter she became more anæmic as well as slightly jaundiced and cyanosed.

She was admitted to hospital on January 16th 1950. Her blood was definitely brownish in colour and formed an opaque solution (due to the suspended Heinz bodies) when added to distilled water. There were 2,[illegible]00,000 erythrocytes per c.mm., 8.0 g. of hæmoglobin per 100 ml. and 53 to 60% reticulocytes; the M.C.V. averaged 130 c.μ. and the M.C.H. concentration 26%.

Romanowsky stained blood films were remarkable for the intensity of punctate basophilia, both diffuse and of the Pappenheimer type, and for the presence of numerous extremely crenated and contracted corpuscles, many of which contained visible pale lilac staining bodies (Fig. 92). The span of erythrocyte osmotic fragility was considerably increased; lysis commenced in 0.55% NaCl (the M.C.F. being 0.41%) NaCl but was not complete in 0.20% NaCl. The rate of autohæmolysis was markedly increased; there was 1% lysis after four hours and 14% lysis after 24 hours' incubation. The serum bilirubin concentration ranged between 1.4 and 1.7 mg. per 100 ml.

Bone marrow biopsy revealed an intensely hyperplastic, predominantly erythropoietic marrow. A large amount of iron-containing pigment was present in phagocytic reticulum cells.

At this point it was discovered that the patient had been dosing herself with undisclosed amounts of acetylphenylhydrazine and also with thiouracil, dicoumarol and sulphasuxidine. She rapidly recovered from her anæmia and the Heinz bodies quickly disappeared from the circulation when she was prevented from taking any further drugs (Fig. 91). When last seen in April 1950 it was clear, however, that she had commenced to drug herself once more: her blood contained 1[illegible]% Heinz bodies and the reticulocyte count was 10%.

Summary. A case of severe hæmolytic anæmia for which splenectomy had been performed. The patient was ultimately found to be drugging herself with acetylphenylhydrazine. Temporary cure followed the withholding of the drug.

Naphthalene

Naphthalene has for long been known to be a potentially hæmolytic substance (Heine, 1913). Recently Zuelzer and Apt (1949) described the consequences of the ingestion of naphthalene balls (moth balls) by four young children. An acute illness characterized by nausea, diarrhœa and fever followed, and anæmia developed acutely. Three of the children had hæmo-

bilirubin concentrations (0 75 to 1 3 mg per 100 ml) In four patients there was evidence of an increased excretion of fæcal urobilinogen Erf and Rhoads referred to the observations of earlier workers which were also suggestive of increased hæmolysis (see also Hunter 1943 and André and Dreyfus 1951)

Nitrobenzene

Intracorpuscular methæmoglobin formation is a characteristic feature of poisoning by nitrobenzene In grave poisoning hæmolysis with jaundice and splenomegaly also develop (Hunter 1943)

Nabarro (1948) described in detail the history of a patient a young woman aged 19 years who took mononitrobenzene by mouth She developed a blue grey cyanosis and passed dark urine and within six days became severely anæmic with hæmoglobinæmia methæmalbuminæmia and hæmoglobinuria Erythrocyte osmotic fragility was slightly increased and there was marked anisocytosis poikilocytosis and punctate basophilia Nabarro mentioned that the dark urine of nitrobenzene poisoning was caused by the presence of para amidophenol as well as by hæmoglobin derivatives

Acetanilide and Phenacetin

Chronic acetanilide intoxication results in cyanosis and an anæmia which is probably hæmolytic in type (Meulengracht and Lundsteen 1939–40) A similar anæmia may be caused by chronic overdosage with phenacetin (Ja[illegible]inski and Muller 19[illegible]0) Small numbers of Heinz bodies may be found in the peripheral circulation

Promin

The sulphone derivative promin has been used in the treatment of tuberculosis and leprosy Hall Pfuetze Hinshaw and Feldman (1942) treated 70 patients the majority receiving daily oral doses of from 1 6 g to 3 2 g In most instances the drug was tolerated for eight to ten days without any marked effect on the blood picture thereafter anæmia developed more or less rapidly

In cases in which the fall was rapid a predominantly neutrophil leukocytosis developed and in some patients a leukæmoid picture was noted

One representative case was described in which the hæmoglobin fell from 66 % to 35% in 10 days and the total leucocyte count reached [illegible] 000 per c mm on withholding the promin the reticulocyte count rose to 58% and a rapid recovery ensued One of the patients appeared to be unusually sensitive to the drug for after only three doses of 1 6 g an acute hæmolytic anæmia with hæmoglobinuria developed as the result of which the hæmoglobin fell to [illegible]0%

Higgins (1943) showed that promin regularly produces anæmia in

globinuria Examination of their blood revealed an acute hæmolytic anæmia with a high leucocytosis and erythroblastæmia Many spherocytes were present as well as a number of fragmenting erythrocytes with irregular and jagged outlines osmotic fragility was markedly *increased The blood plasma was brownish in* colour and contained free hæmoglobin and methæmalbumin

Heinz bodies were observed in one case but intracorpuscular methæmoglobin and sulphæmoglobin were not found Zuelzer and Apt made the point that in any case of acute hæmolytic anæmia in childhood the possibility of the ingestion of moth balls should be considered

Schafer (1951) described a fatal hæmolytic anæmia in a newborn infant which he considered might have been due to the absorption through the skin of naphthalene which had been used to impregnate the infant s napkins

A further case was published by Mackell Rieders Brieger and Bauer (1951) α and β naphthol and α and β naphthoquinone were isolated from the baby s urine Mackell and co workers also carried out *in vitro* and *in vivo* tests (in rabbits) with naphthalene and its degradation products They found that the chemicals could be ranged in respect of their hæmolytic potency in the following order α naphthol which was most hæmolytic β naphthol the naphthoquinones and finally naphthalene which was least hæmolytic

β Naphthol

β naphthol has been used in the treatment of hookworms It also is a potentially hæmolytic drug Out of 70 patients given large doses of the drug (18 g in adults) four developed acute hæmolytic episodes with hæmoglobinuria (*Smillie 1920*) Irregularly shaped erythrocytes were observed in one patient and marked punctate basophilia in two others A leucocyte count of 40 000 per c mm was recorded in a patient whose hæmoglobin had fallen as low as 22%

Trinitrotoluene

Minot (1919) studied the blood of a large series of munition workers exposed to trinitrotoluene He stressed the frequency of excess polychromasia and observed in some instances that the erythrocytes were undergoing fragmentation in the peripheral blood

Benzene

Benzene poisoning probably causes increased blood destruction as well as hypoplasia or aplasia of the bone marrow Erf and Rhoads (1939) studied nine anæmic patients who gave a history of exposure to benzene eight of the patients recovered—one died of *myeloid leukæmia In four patients marrow biopsy* showed hyperplasia not hypoplasia All the patients had raised reticulocyte counts (3 7 to 14%) and slightly raised plasma

bilirubin concentrations (0·75 to 1·3 mg per 100 ml). In four patients there was evidence of an increased excretion of fæcal urobilinogen. Erf and Rhoads referred to the observations of earlier workers which were also suggestive of increased hæmolysis (see also Hunter, 1943, and André and Dreyfus, 1951).

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In cases in which the fall was rapid a predominantly neutrophil leukocytosis developed and in some patients a "leukæmoid" picture was noted.

One representative case was described in which the hæmoglobin fell from 66% to 35% in 10 days and the total leucocyte count reached 22,000 per c.mm.; on withholding the promin the reticulocyte count rose to 58% and a rapid recovery ensued. One of the patients appeared to be unusually sensitive to the drug, for after only three doses of 1·6 g. an acute hæmolytic anæmia with hæmoglobinuria developed, as the result of which the hæmoglobin fell to 20%.

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from 0·025 g to 0·1 g of lead. The erythrocyte count of a susceptible patient fell abruptly within a half to one hour of the injection and as much as half of his erythrocytes might be destroyed as the result of a single dose. In such patients a proportion of the erythrocytes was noted to be markedly distorted; many cells were folded and some showed small indentations as if pieces had been bitten out of them with a punch. Punctate basophilia developed during the regeneration phase, the rise in stippled cells paralleling the reticulocyte count. As a rule the acute hæmolytic episodes were associated with a rise in the platelet count.

Less dramatic changes were reported by Gould, Kullman and Sheeket (1937) in patients receiving courses of colloidal lead triphosphate by intravenous injection. Anæmia did not usually become obvious until after two to three weeks of treatment. Normoblasts were then found in the peripheral blood, the cytoplasm of many of them being stippled. Large numbers of stippled erythrocytes also appeared in the blood, their number being only slightly less than that of the reticulocytes. A predominantly neutrophil leucocytosis developed in most cases.

In chronic lead poisoning anæmia is less noticeable and rarely severe. Punctate basophilia is probably invariable (Lane, 1931); it has been widely used as a diagnostic aid in lead intoxication (Machle, 1947). Another characteristic finding is the presence of coproporphyrin III in the urine. Maloof (1950) contended that coproporphyrin in the urine might be a more sensitive index of lead poisoning than the stippled cell count. Neither finding is, of course, a specific sign of lead poisoning.

Pathogenesis. The anæmia of lead poisoning is probably brought about by the chemical affecting hæmopoiesis in the marrow as well as damaging circulating erythrocytes.

The evidence for a direct hæmolytic effect in patients receiving large doses of lead has already been referred to (Brookfield, 1928), the morphological signs of erythrocyte damage being comparable with those produced by phenylhydrazine (Fig. 2, p. 12).

The effects of lead on hæmopoiesis need further consideration. It is generally agreed that the stippled cell count runs parallel with the reticulocyte count, although as a rule at a slightly lower level, and that the stippled cell is an immature cell (a reticulocyte), the basophilic ribonucleoprotein of which has been slightly altered as the result of lead intoxication. This change is also noticeable in the nucleated erythrocyte precursors.

Stippling of normoblasts was noted by Bell, Williams and Cunningham (1925), by Gould, Kullman and Sheeket (1937) and by Klima and Seyfried (1937). The phenomenon was studied in detail by McFadzean

guinea pigs as well as causing the formation of intracorpuscular methæmoglobin and sulphæmoglobin The drug appears to affect the erythrocyte surfaces for within a few hours of its administration many crenated cells could be seen in wet preparations and in films No true spherocytosis resulted and the corpuscles were found to have become unusually resistant to hæmolysis in hypotonic saline

Diaminodiphenylsulphone

The onset of a probable hæmolytic anæmia was reported by Ramanujam and Smith (1951) in a patient receiving treatment for leprosy

Methylchloride

Methylchloride which is widely used as a refrigerant in domestic refrigerators is a potential cause of hæmolytic anæmia Three cases of poisoning were reported by Kegel McNally and Pope (1929) The lowest hæmoglobin recorded was 50% and the highest bilirubin concentration 2 2 mg per 100 ml Three days after the onset of the illness anisocytosis and fragmentation of the erythrocytes were noted

Inorganic Chemicals

Arsine

Inhalation of arsine is a well known cause of hæmolytic anæmia in man (Dudley 1919 Bomford and Hunter 1932 Hunter 1943) and experimental animals (Kiese 1937) Intravascular hæmolysis takes place within a few hours of exposure and hæmoglobinuria follows Jaundice develops and the erythrocyte count may fall within the next day or two to very low levels In non fatal cases basophilic stippling is often conspicuous during the phase of regeneration Kiese (1937) reported a marked increase in erythrocyte osmotic fragility as the result of the inhalation of arsine by dogs

Lead Poisoning

The toxic effect of lead on the blood has been known for many years and many clinical and experimental studies have been carried out The early literature is reviewed by Aub Fairhall Minot and Reznikoff (1925) *In vitro* studies (see Aub *et al* 1925) have shown that lead has a direct effect on the surfaces of the erythrocytes the cells undergo contraction and become inelastic and brittle they break up readily as the result of mechanical trauma and undergo rapid spontaneous lysis although appearing on the whole more resistant to osmotic hæmolysis

In man acute lead poisoning may produce dramatic effects on the blood Brookfield (1928) studied the blood of patients who were being treated for malignant disease by intravenous doses of

probably hæmolytic in origin Watson and Spink (1940) also noted that the anæmia tended to be hypochromic and suggested that hæmoglobin formation was also affected Collateral evidence for this was provided by the finding of relatively large amounts of coproporphyrin III in the urine (Rimington 1938 Figge Carey and Weiland 1946) and an increase in erythrocyte porphyrin (Watson 1950)

Intracorpuscular methæmoglobin and sulphæmoglobin are also produced by sulphonamide therapy (Harris and Michel 1939) sulphanilamide is the most active drug in this respect sulphapyridine less active and sulphathiazole the least active of the three Moeschlin (1940) reported yet another effect the production of Heinz bodies by a patient treated with sulphapyridine Subsequent observations on mice (Moeschlin 1941–42) again showed that sulphanilamide was the most potent of the three drugs in producing Heinz bodies and sulphathiazole the least potent

The effects of the drugs on experimental animals were studied by Richardson (1939 1941) Machella and Higgins (1939) and by Antopol Goldman and Sampson (1941) Richardson (1941) working with mice and using the development of anæmia and cyanosis (due to sulphæmoglobin) as criteria showed that although sulphanilamide was more injurious than sulphapyridine (2 1 times) and sulphapyridine more injurious than sulphathiazole (4 3 times) when allowances were made for differences in absorption and excretion their respective toxicities did not differ greatly Cyanosis was observed only when large doses of sulphanilamide or sulphapyridine were given Heinz bodies were present in small numbers even in mildly anæmic mice the number present increasing roughly parallel to the degree of anæmia Antopol and co-workers (1941) reported that a moderately severe anæmia associated with splenic enlargement regularly developed in rats and that osmotic fragility was slightly decreased

Acute Hæmolytic Anæmia in Man due to Sulphonamide Drugs

It seems likely that the episodes of acute hæmolysis which have occurred in the course of therapy with sulphonamide drugs are due to the patients hypersensitivity Hæmolysis usually takes place at an early stage of treatment as a rule within 24 to 72 hours of taking the drugs Moreover the onset of hæmolysis cannot be correlated with excessive dosage and second attacks may be precipitated by the re administration of the drug to the same patient (Wood 1938 Fox and Ottenberg 1941)

The first cases of acute hæmolytic anæmia following the use of sulphanilamide were described by Harvey and Janeway (1937)

and Davis (1949) who showed in man and in guinea pigs experimentally poisoned with lead salts that the granules of stippled normoblasts contained free ionized iron which gave a positive Prussian blue reaction with acid ferrocyanide They noted that hæmoglobin formation was grossly deficient in the cells which contained the largest granules They also demonstrated that many of the stippled cells in the peripheral blood were in fact siderocytes McFadzean and Davis (1949) and Pirrie (1952) found that when guinea pigs poisoned with lead were splenectomized a great increase in the number of stippled cells in the blood followed They suggested that in lead intoxication the defective stippled erythrocytes were removed from the circulation by the spleen in man and in the normal (non splenectomized) animal and probably also by reticuloendothelial cells elsewhere and that this was an additional factor in the causation of the anæmia

Lead also interferes with the formation of the porphyrin precursors of hæmoglobin Coproporphyrin III is formed in increased amounts and is excreted in the urine (Grinstein Wikoff de Mello and Watson 1950) and there is also an increase in erythrocyte coproporphyrin (Watson 1950)

HÆMOLYTIC ANÆMIA DUE TO HYPERSENSITIVITY TO DRUGS OR CHEMICALS

Hæmolytic Anæmia due to the Sulphonamides

The toxic effects of the sulphonamide drugs have been extensively studied perhaps the most serious is acute hæmolytic anæmia Its incidence is very low at the present time due to the fact that drugs of the sulphonamide group are now administered much less often than they were in the period between 1937 and 1944 and also because sulphanilamide by far the most dangerous is now practically never used at all

Most of the early observations on clinical and experimental toxicity were carried out with sulphanilamide and to a lesser extent with sulphapyridine and sulphathiazole It was soon realized that a slight degree of progressive anæmia frequently followed the administration of the drugs (Jennings and Southwell Sander 1937) The anæmia was more marked with sulphanilamide than with sulphapyridine or sulphathiazole and its intensity was more or less correlated with the amounts of drug given (This type of anæmia is quite distinct from the rarer and more dramatic acute hæmolytic anæmia considered in the next section which is not in any way connected with dosage)

Watson and Spink (1940) and Erf and Macleod (1940) nevertheless demonstrated that the simple benign anæmia was accompanied by an increased excretion of urobilinogen in the fæces i e it was

detectable on the next Stats Wasserman and Rosenthal (1948) found fragmenting erythrocytes in the peripheral blood of a patient suffering from acute hæmolytic anæmia following sulpha pyridine therapy During the early regenerative phase erythro blastæmia may be marked and during recovery conspicuous polychromasia and a high reticulocytosis are commonly found

Oxyhæmoglobin is often present in large amounts in the patients sera during the height of the hæmolysis later increases in serum bilirubin concentration usually result in clinical jaundice The prompt van den Bergh reaction may be positive in some instances indicating concurrent liver damage Intracorpuscular methæmo globin or sulphæmoglobin cannot usually be demonstrated in the acute hæmolytic anæmias due to the sulphonamides—the abnormal pigments only develop when large doses of the drugs have been given

A high leucocytosis is characteristic Total counts of 50 000 cells per c mm or more are not infrequent (Harvey and Janeway 1937 Spence and Roberts 1940 Fox and Ottenberg 1941 Keefer 1942 Ross and Taegel 1946) The majority of the leucocytes are neutrophils but myelocytes are also usually present

Hæmoglobinuria is usually found in the most severely affected patients Oliguria and nitrogen retention due to renal failure have also been reported (Myers and Rom 1940 Spence and Roberts 1940)

Serology Abnormal antibodies which can be attributed with any degree of certainty to the drugs do not seem to have been detected in sulphonamide hæmolytic anæmia (see also *Patho genesis* p 396)

Prognosis and Treatment The outlook is serious The mortality from acute hæmolytic anæmia following sulphonamide therapy was between 5 and 10% The cause of death has been complex in most cases with acute anæmia renal failure and the primary disease for which the drug was given acting in combina tion Treatment consists of withholding the drug and giving blood transfusions and appropriate treatment for renal failure if present

Pathogenesis As already mentioned on p 393 an undue susceptibility seems to be the cause of acute hæmolytic anæmia following sulphonamide therapy Emerson Ham and Castle (1941) advanced the hypothesis that the capricious hæmolytic activity *in vivo* of compounds such as sulphanilamide was due to the formation in certain patients of unusual metabolites which were powerfully hæmolytic Compounds such as hydroquinone

two of their patients had throat infections and another meningococcal meningitis. Their hæmoglobin concentrations fell precipitously after 36 hours to seven days of therapy; one patient had taken only 4.8 g of the drug.

A large series of cases was subsequently reviewed by Wood (1938) of 522 patients with various acute or chronic infections treated with sulphanilamide: 8.3% of the children and 2.4% of the adults developed acute hæmolytic anæmia. The onset was usually within 24 to 72 hours of taking the drug, the maximum degree of anæmia developing between the third and seventh day. Four out of five patients relapsed when the drug was re-administered. The patients who developed anæmia were not given more than the usual doses nor were their blood sulphanilamide levels abnormally high.

Long, Bliss and Feinstone (1939) reported almost exactly the same incidence of hæmolytic anæmia, i.e. 2.9% of 307 adults and 8.9% of 101 children. Long, Haviland, Edwards and Bliss (1940) reviewed a large series of patients treated with different sulphonamide compounds: 1.8% of the patients treated with sulphanilamide and 0.6% treated with sulphapyridine developed acute hæmolytic anæmia; this was not seen in any of the patients treated with sulphathiazole. In a later review Keefer (1942) put the incidence of acute hæmolytic anæmia following sulphapyridine as at least as high as that following the use of sulphanilamide. Cases of acute hæmolytic anæmia following sulphathiazole were said to be very rare.

Many good clinical descriptions are available with detailed laboratory findings of acute hæmolytic anæmia following the administration of sulphanilamide or sulphapyridine (e.g. Spence and Roberts, 1940; Fox and Ottenberg, 1941). A detailed report of acute hæmolytic anæmia following sulphadiazine therapy with references to several other possible cases was published by Ross and Paegel (1946).

Laboratory Findings. The patients often become severely anæmic. The hæmoglobin falls as a rule to less than 7.5 g per 100 ml, with a corresponding reduction in the erythrocyte count. Sometimes half the patient's erythrocytes are destroyed in 24 hours and this results in intense hæmoglobinæmia and hæmoglobinuria. Marked spherocytosis has been observed in some patients at least in the early stages of the acute hæmolytic phase (Ham and Castle, 1940; Gilligan and Kapnick, 1941; Ross and Paegel, 1946). Other authors reported that osmotic fragility was normal (Harvey and Janeway, 1937; Myers and Rom, 1940). Ross and Paegel (1946) attributed these negative findings to the fact that the tests were carried out relatively late in the course of the disease, i.e. after the first acute hæmolytic phase had ended. Ross and Paegel demonstrated how transient the increase might be: in their patient it was strikingly obvious one day and hardly

Earle Bigelow Zubrod and Kane (1948) treated 157 patients with daily doses of at least 30 mg of pamaquin seven patients developed acute anæmia with hæmoglobinuria usually on the third to fifth day of treatment Earle and co-workers concluded that negroes were more susceptible than white patients and that there appeared to be no correlation between the plasma pamaquin concentration and the development of hæmolysis they considered that previous quinine therapy might have been a contributory factor Methæmoglobin was formed more regularly and this could be correlated with dosage and the concentration of pamaquin in the plasma Rosenfield Zubrod Blake and Shannon (1948) found that methæmoglobin was formed more readily if quinine was given at the same time as pamaquin

Recently Dern and co workers (1954) made the interesting observation that susceptibility to hæmolysis by primaquine resided in the susceptible subjects erythrocytes They found that the erythrocytes of a susceptible subject tagged with radio chromium and transfused into a non sensitive subject were hæmolysed when primaquine was given to the recipient and that normal (non sensitive) corpuscles were not hæmolysed when transfused to a sensitive subject to whom primaquine had been given

Para aminosalicylic Acid (P A S)

A small number of cases have been reported mostly in children of acute hæmolytic anæmia following the oral administration of the sodium salt of P A S (Janet Weill Haquin and Harl 1951 Christiaens and Goudemand 1952) In most instances hæmolysis commenced abruptly within a day or two of starting treatment

The cause and mechanism of the anæmia is obscure The possibility that a powerfully hæmolytic degradation product had been formed from the P A S was discussed by Christiaens and Goudemand but no positive evidence for this could be obtained It was concluded that an abnormal susceptibility was the more likely explanation

Cryogénine (Phenylsemicarbazide)

This antipyretic drug has been responsible for a number of episodes of acute hæmolysis Most of the case reports are to be found in the French literature (e g Marie *et al* 1950 see also Oltramare 1953) Young subjects seem to be particularly sensitive Phenylsemicarbazide is a chemical not very different from phenylhydrazine and Marie and his colleagues suggested that the hæmolysis might be brought about by the degradation of the drug into hæmolytic metabolites of which phenylhydrazine might be one

and para aminophenol which form oxidants in oxidation reduction systems were found to cause an increase in osmotic fragility and ultimately hæmolysis *in vitro* It was also found that these compounds when administered to cats caused an acute hæmolytic anæmia with increase in osmotic fragility Sulphanilamide itself was inactive *in vitro* and *in vivo* in equivalent dosages The suggestions of Emerson Ham and Castle are attractive but it has yet to be shown exactly what are the derivative or derivatives of the sulphonamides which are hæmolytic *in vivo* in man

The presence of auto agglutinins has been reported in some instances and Dameshek (1943) suggested that these might have been formed as the result of an alteration in erythrocyte antigenicity due in some way to the action of the drug (see also p 293) However it now appears probable in nearly all the instances in which auto agglutinins were observed that the patients were suffering from hæmolytic anæmia following virus pneumonia Cases of this type were published by Antopol Applebaum and Goldman (1939) Rothstein and Cohn (1942) Dameshek (1943) Donald and Wunsch (1944) and Layne and Schemm (1944) It is interesting to note that in these patients the anæmia occurred between the tenth and eighteenth day of their illness the latent period being longer than is usual in the hæmolytic anæmias due to drug sensitivity

Hæmolytic Anæmia due to Hypersensitivity to Drugs or Chemicals other than the Sulphonamides

Quinine

There are a small number of recorded instances of acute hæmolytic anæmia apparently due to sensitivity to quinine Most of these seem to have occurred in patients who have taken large doses of the drug in order to produce abortion (Terplan and Javert 1936 Licciardello and Stanbury 1948)

Pamaquin (plasmochin) and primaquine

Acute hæmolytic anæmia is a well known complication of the treatment of malaria with pamaquin and similar drugs Hardgrove and Applebaum (1946) referred to 72 patients with hæmoglobinuria and Dimson and McMartin (1946) observed thirteen examples of hæmolytic anæmia and hæmoglobinuria amongst 10 000 Indian troops receiving mepacrine quinine and small doses (maximum 0 15 g) of pamaquin three of the patients died Dimson and McMartin concluded that the concurrent or previous use of mepacrine was a contributory factor

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Para aminosalicylic Acid (P A S)

A small number of cases have been reported mostly in children of acute hæmolytic anæmia following the oral administration of the sodium salt of P A S (Janet Weill Haquin and Harl 1951 Christiaens and Goudemand 1952) In most instances hæmolysis commenced abruptly within a day or two of starting treatment

The cause and mechanism of the anæmia is obscure The possibility that a powerfully hæmolytic degradation product had been formed from the P A S was discussed by Christiaens and Goudemand but no positive evidence for this could be obtained It was concluded that an abnormal susceptibility was the more likely explanation

Cryogénine (Phenylsemicarbazide)

This antipyretic drug has been responsible for a number of episodes of acute hæmolysis Most of the case reports are to be found in the French literature (e g Marie *et al* 1950 see also Oltramare 1953) Young subjects seem to be particularly sensitive Phenylsemicarbazide is a chemical not very different from phenylhydrazine and Marie and his colleagues suggested that the hæmolysis might be brought about by the degradation of the drug into hæmolytic metabolites of which phenylhydrazine might be one

and para aminophenol which form oxidants in oxidation reduction systems were found to cause an increase in osmotic fragility and ultimately hæmolysis *in vitro* It was also found that these compounds when administered to cats caused an acute hæmolytic anæmia with increase in osmotic fragility Sulphanilamide itself was inactive *in vitro* and *in vivo* in equivalent dosages The suggestions of Emerson Ham and Castle are attractive but it has yet to be shown exactly what are the derivative or derivatives of the sulphonamides which are hæmolytic *in vivo* in man

The presence of auto agglutinins has been reported in some instances and Dameshek (1943) suggested that these might have been formed as the result of an alteration in erythrocyte antigenicity due in some way to the action of the drug (see also p 293) However it now appears probable in nearly all the instances in which auto agglutinins were observed that the patients were suffering from hæmolytic anæmia following virus pneumonia Cases of this type were published by Antopol Applebaum and Goldman (1939) Rothstein and Cohn (1942) Dameshek (1943) Donald and Wunsch (1944) and Layne and Schemm (1944) It is interesting to note that in these patients the anæmia occurred between the tenth and eighteenth day of their illness the latent period being longer than is usual in the hæmolytic anæmias due to drug sensitivity

Hæmolytic Anæmia due to Hypersensitivity to Drugs or Chemicals other than the Sulphonamides

Quinine

There are a small number of recorded instances of acute hæmolytic anæmia apparently due to sensitivity to quinine Most of these seem to have occurred in patients who have taken large doses of the drug in order to produce abortion (Terplan and Javert 1936 Licciardello and Stanbury 1948)

Pamaquin (plasmochin) and primaquine

Acute hæmolytic anæmia is a well known complication of the treatment of malaria with pamaquin and similar drugs Hardgrove and Applebaum (1946) referred to 72 patients with hæmoglobinuria and Dimson and McMartin (1946) observed thirteen examples of hæmolytic anæmia and hæmoglobinuria amongst 10 000 Indian troops receiving mepacrine quinine and small doses (maximum 0 15 g) of pamaquin three of the patients died Dimson and McMartin concluded that the concurrent or previous use of mepacrine was a contributory factor

HEINZ BODY ANÆMIA

The presence of Heinz bodies in erythrocytes has been referred to as a common accompaniment of intoxication with certain chemicals and drugs e g phenylhydrazine acetylphenylhydrazine (pyrodin) nitrobenzene aniline sulphapyridine etc A bibliography is given by Webster (1949) There are however patients whose blood contains Heinz bodies the presence of which cannot be explained by poisoning with any exogenous chemical or drug Such patients may be referred to as suffering from spontaneous Heinz body anæmia

Spontaneous Heinz body (Innenkörper) Anæmia

In Premature Infants Willi (1947) reported Heinz bodies in 49 5% of the erythrocytes of an infant who was being treated for otitis with the sulphanilamide derivative Elkosin He also referred to the presence in a newborn mongol of large numbers of Heinz bodies for which no satisfactory cause could be found Willi and Hartmeier (1940) subsequently carried out a detailed investigation into the incidence of Heinz bodies in the blood of newborn infants Small numbers of the bodies were found in 277 out of 1 251 infants investigated the great majority of the infants (81 4%) with more than 2 1% of affected corpuscles being premature or underweight The cause of the Heinz body formation could not be established in particular there seemed to be no relationship between their presence and the administration of drugs to the mother before or at the time of birth The percentage of corpuscles containing Heinz bodies increased as a rule after birth and was maximal between the third and seventh day The presence of the Heinz bodies did not seem to be associated with anæmia

Gasser and Karrer (1948) and Gasser (1951) however have described newborn infants in whom marked Heinz body development was associated with hæmolytic anæmia of unknown origin Gasser (1953) has recently published a full account of this syndrome based on fourteen cases All his patients were premature or underweight full term infants jaundice was noticeable on the first day and was often prolonged and they all tended to develop an anæmia which was maximal as a rule in the third week Up to 45% of the corpuscles contained Heinz bodies and these were present in the largest numbers from about the fifth to the tenth day The reticulocyte count was low at the time of maxi

Phenothiazine

There appears to be a definite risk of acute anæmia developing as the result of taking phenothiazine in therapeutic doses DeEds Stockton and Thomas (1939) treated with the drug 49 patients suffering from urinary infections anæmia was noted in three of the patients all of whom had been given unusually large doses (average dosage 23 3 g)

The patient who was most severely affected had taken 19 9 g in eight days Anæmia was noticed on the seventh day and this increased in severity until the 14th day when the erythrocyte count was found to be 1 ,30 000 cells per c mm Fifty per cent of the erythrocytes were said to be fragmented but the osmotic fragility was reported to be normal The leucocyte count rose to 21 650 cells per c mm A further example of acute hæmolytic anæmia was reported by Johnstone (1942) The patient was a child who had been given 10 g of the drug as treatment for threadworms he recovered after his hæmoglobin had fallen to 42%

Neoarsphenamine

Young Valentine and Howland (1946) reported an instance of fatal hæmolytic anæmia apparently due to sensitivity to neoarsphenamine They could find no similar instance in the literature An acute hæmolytic episode occurred shortly after the patient had received his fifth injection (0 4 g) of the drug There was marked hæmoglobinæmia hæmoglobinuria and mild spherocytosis Oliguria supervened and the patient became jaundiced At autopsy focal areas of necrosis were seen in the liver the kidneys contained pigment casts and the spleen was intensely congested

Benzedrine

Hæmolytic anæmia possibly due to sensitivity to benzedrine has also been reported It is clearly a very rare event Davies (1937) described the case of a man who took 190 mg of the drug in 19 days At the end of this time he fainted and was found to have only 40% hæmoglobin One week later his hæmoglobin was 56% and polychromasia was noted in the stained blood film The patient was considered to have been in good health previously

Mesantoin

Snapper Marks Schwartz and Hollander (1953) observed a patient who developed hæmolytic anæmia whilst taking the anti epileptic drug mesantoin The antiglobulin test became positive and abnormal antibodies were demonstrated in the patient s serum The exact role of the drug in relation to the anæmia could not be determined

Antihistamines

Diphenylhydramine and pyribenzamine have both been considered to have caused hæmolytic anæmia (Crumbley 1950) This claim needs confirmation

Effect of Splenectomy on Heinz body Formation

In the author s Case 31 and Cathie s (1952) patient very large numbers of large Heinz bodies were present in the peripheral blood—both patients had undergone splenectomy It is probable that removal of the spleen was responsible for the exceptionally large numbers of circulating Heinz bodies

There is experimental evidence that splenectomy in animals results in an increased number of Heinz bodies in the peripheral blood (Webster 1949) and in man too Heinz bodies have been detected in small numbers in the peripheral blood of some patients after splenectomy as well as in patients with splenic hypoplasia (Zadek and Burg 1930 Selwyn and Mollin 1954) Selwyn and Mollin found that if splenectomized patients were given small doses of phenacetin Heinz bodies could be regularly found in the peripheral blood

It is probable that even in healthy adults there is a tendency to form Heinz bodies and that (?) normal metabolites are capable of denaturing the hæmoglobin in much the same way perhaps as chemicals such as phenacetin The normal spleen however seems to have the capacity either of removing Heinz bodies from erythrocytes or of filtering off from the circulation cells containing the bodies

Staining Properties of Heinz Bodies

Mention was made on p 387 of the pale lilac staining ring like structures corresponding in size to Heinz bodies which were seen in Romanowsky stained blood films of the patient suffering from acetyl phenylhydrazine poisoning Exactly the same structures could be seen in stained films of Cathie s patient who was suffering from Heinz body anæmia of apparently endogenous origin This finding was unexpected as Heinz bodies do not normally stain by Romanowsky dyes in blood films fixed in methanol

Both the patients referred to in the previous paragraph had undergone splenectomy However it does not seem likely that splenectomy *per se* was responsible for the unusual staining Selwyn (1954) studied the staining properties of Heinz bodies produced in rabbits as the result of the administration of acetylphenylhydrazine both before and after splenectomy He found exactly the same staining appearances as seen in the human cases if the drug was given to anæmic rabbits with high reticulocyte counts irrespective of whether their spleens had been previously excised He concluded that the peculiar staining appearance was due to the action of acetylphenylhydrazine on immature erythrocytes

Chronic Hæmolytic Anæmia with Methæmoglobinæmia and Sulphæmoglobinæmia

Evans Enzer Eder and Finch (1950) described two patients who suffered from anæmia and episodes of cyanosis pain in the chest and abdomen and syncope The anæmia appeared to be hæmolytic in type and the cyanosis was shown to be due to intra

mum Heinz body formation but it rose subsequently and exceeded 6% in most instances Contracted and distorted erythrocytes were a conspicuous feature of blood films made when the Heinz bodies were present in their greatest numbers No cause for the Heinz bodies could be found However Gasser suggested that their formation might be due to a difficulty in adaptation in *premature infants perhaps associated with dysfunction of the* spleen or in the mechanisms which normally protect erythrocytes from damage

In Older Children Cathie (1952) described a remarkable example in a child of a congenital anæmia associated with the formation of numerous Heinz bodies (at least after splenectomy)

The child was born five weeks prematurely and had always been pale and jaundiced He was first investigated in hospital when 16 months of age His hæmoglobin was 7 g per 100 ml and there were 37% reticulocytes Erythrocyte osmotic fragility was increased Hæmolytic anæmia was diagnosed and splenectomy performed in June 1948 after a preliminary blood transfusion Four months later the child was readmitted with a recurrence of anæmia 75% of his erythrocytes *being reticulocytes*

In January 1950 many of his corpuscles were found to contain large Heinz bodies and Romanowsky stained films showed almost exactly the same appearance as those of the author s patient (Case 31 Fig 92) who was suffering from acetylphenylhydrazine poisoning (after splenectomy) Many of the corpuscles were crenated and shrunken and some of these contained recognizable palely basophilic structures of the size of Heinz bodies Punctate basophilia and diffuse poly chromasia were striking features and Pappenheimer bodies were numerous No cause for the development of the Heinz bodies could be found The child was kept under strict observation but the Heinz bodies did not disappear and it seemed impossible that he was taking or being given any noxious chemical or drug

In Adults Fertman and Doan (1948) reported an example of anæmia with Heinz bodies of possibly endogenous origin in an elderly man

The patient was a physician who had taken ½ gr erythrol tetranitrate tablets as treatment for angina pectoris during the year before his admission into hospital for the investigation of anæmia

His peripheral blood contained bizarre shaped poikilocytes and Heinz *bodies were noted in 1 to 16% of the erythrocytes* The erythrol tetranitrate was discontinued but the anæmia was progressive and the numbers of Heinz bodies did not decrease In this case therefore it is at least possible that the Heinz body formation was due to some acquired internal metabolic derangement rather than due to the erythrol tetranitrate

septicæmia (Mollison 1947 Hagberg 1952) and in *H influenzæ meningitis* (Cerversman and Hoo 1951) Cusser (1951) described several examples of hæmolytic anæmia in children suffering from *endocarditis* due to *streptococci* or *staphylococci* and in *pneumococcal pyæmia* Acute hæmolytic episodes with hæmoglobinuria have also been reported as rare complications of *typhoid* (Shaw 1951 McFadzean and Choa 1953) and in *cholera* (De Sengupta and Chanda 1954)

Certain bacteria or bacterial toxins regularly cause hæmolytic anæmia *Cl welchii septicæmia* usually following abortion is a well known cause of hyperacute hæmolysis and *Bartonella* infection in man (oroya fever) may be quoted as a further example (Ricketts 1948 1949)

The mechanism of hæmolysis is obscure in many of the cases of hæmolytic anæmia accompanying infections When hæmolysis is a regular consequence of the infection as in *Cl welchii* septicæmia a direct effect of the bacterial toxin on the erythrocytes is probable However in infections in which overt hæmolysis is a rare event an unusual susceptibility of the patient must be postulated

Slight degrees of increased hæmolysis not sufficient to cause clinical signs of hæmolysis are probably much more frequent in infections than is overt hæmolytic anæmia

Schlegel and Bottner (1951) for instance found the survival of transfused normal erythrocytes to be slightly impaired in ten patients with bacterial endocarditis and in six patients with tuberculosis Survival studies have also demonstrated an increased rate of erythrocyte elimination in rheumatoid arthritis (Mollison and Patterson 1949) and in rheumatic fever during exacerbations of the disease (Rheinhold 1954) The direct antiglobulin tests were negative in the above mentioned cases of rheumatoid arthritis and rheumatic fever Zoutendyk and Gear (1951) however have recorded positive reactions in four out of ten patients with acute rheumatic fever

Hæmolytic Anæmia in Protozoal Infections

De Vries (1946) studied ten patients with malaria (none had blackwater fever) and found that the urobilinogen excretion in the fæces was raised in all of them In kala azar too excessive hæmolysis seems to be an important cause of anæmia in certain patients (Burchenal Bowers and Haedicke 1947 Rachmilewitz de Vries and Gurevitch 1952)

Hæmolytic Anæmia in Vitamin Deficiencies

It is possible that certain of the vitamins protect erythrocytes from hæmolysis Experimentally vitamin E protects the

corpuscular methæmoglobin and sulphæmoglobin The syndrome appeared to be similar to that referred to as enterogenous cyanosis In one patient no cause for the abnormal pigment formation (or the anæmia) could be discovered in the other a nitrite forming organism recovered from the urine and from abscesses in the right kidney may have been responsible No mention was made of any morphological abnormalities of the erythrocytes

The author has investigated through the courtesy of Dr Nancy Richardson a patient who presented the same clinical syndrome As in the first case of Evans and co workers the cause of the anæmia and abnormal pigment production could not be established Peripheral blood films of the patient showed markedly distorted and contracted erythrocytes (Fig 3 p 13)

Hæmolytic Anæmia due to Vegetable Poisons

Vegetable poisons are a rare cause of hæmolytic anæmia in man Gasser (1951) mentioned extract of *Filix mas* and mushrooms as possible causes He described an example of acute hæmolytic anæmia with hæmoglobinuria in a one and a half year old child after the ingestion of mushrooms

HÆMOLYTIC ANÆMIA IN ACUTE AND CHRONIC BACTERIAL INFECTIONS

It is difficult to draw any firm conclusions as to the frequency with which overt hæmolytic anæmia or subclinical increased hæmolysis develop as complications of infections particularly as only a few detailed studies using erythrocyte survival techniques seem to have been undertaken However even a superficial study of the literature shows that hæmolytic anæmia has been observed occasionally in a wide range of infections In some patients the anæmia may have been a coincidental but it does not seem likely that this is the explanation in all cases

Excluding virus infections which have been dealt with in Chapter 8 hæmolytic anæmia has been described following or in association with *scarlet fever* by Guneher (1923) (who also referred to earlier reports of hæmoglobinuria in association with tetanus erysipelas scarlet fever typhus pneumococcal sepsis and acute rheumatism) *scarlet fever* and *hilar tuberculosis* (Spira 1943) *pulmonary tuberculosis* (Mollison 1947) *acute areactive tuberculosis* (Lindeboom 1950) *tuberculosis of the spleen* (Hemmeler and Lob 1950) and *miliary tuberculosis* (Plauchu Revol Lejeune and Jouvenceaux 1952)

Hæmolytic anæmia has also been observed in *streptococcal*

septicæmia (Mollison 1947 Hagberg 1953) and in *H influenzæ meningitis* (Cerversman and Hoo 1951) Gasser (1951) described several examples of hæmolytic anæmia in children suffering from *endocarditis* due to *streptococci* or *staphylococci* and in *pneumococcal pyæmia* Acute hæmolytic episodes with hæmoglobinuria have also been reported as rare complications of *typhoid* (Shaw 1951 McFadzean and Choa 1953) and in *cholera* (De Sengupta and Chanda 1954)

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Hæmolytic Anæmia in Vitamin Deficiencies

It is possible that certain of the vitamins protect erythrocytes from hæmolysis Experimentally vitamin E protects the

erythrocytes of rats from hæmolysis by dialuric acid and alloxan (Rose and György 1952) In man the increased hæmolysis in vitamin B_{12} deficiency has been mentioned on p 132 Merskey (1953) has likewise demonstrated increased elimination of transfused erythrocytes in vitamin C deficiency

HÆMOLYTIC ANÆMIA IN BURNS

Ever since the early work of Schultze (1865) it has been realized that heating blood at temperatures above 50° C rapidly produces fragmentation of the erythrocytes the formation of spheroid forms and hæmolysis

The recent careful experimental studies of Ham Shen Fleming and Castle (1948) have defined the sequence of changes and the exact degree of heating necessary to produce them Using human and dog erythrocytes they found that no demonstrable changes followed heating at 46 C for one hour but at 47 to 50 C changes occurred dependent upon the duration of the heating at 51 to 65 C marked changes followed exposure for one to two minutes

The first discernible change produced by heat is the appearance of bud like excrescences from the surfaces of the erythrocytes then multiple buds appear and these become detached Finally the intact erythrocytes and the fragments undergo progressive and irreversible sphering and at this stage their osmotic and mechanical fragilities become markedly increased

Hæmoglobinæmia and hæmoglobinuria have been observed in human cases of severe burning Detailed studies of severely burned patients were reported by Shen Ham and Fleming (1943) *Brown (1944) and Ham Shen Fleming and Castle (1948)* Hæmoglobinæmia and hæmoglobinuria were noted in eleven of the patients of Shen Ham and colleagues and varying degrees of increased erythrocyte osmotic and mechanical fragilities were found in six of them corresponding with the appearance in the peripheral blood of spherocytes and shortly after the burning of small schistocytes The spherocytes had disappeared from the circulation and the osmotic fragility returned to normal within 60 hours in the patients who survived

The studies mentioned in the preceding paragraph refer to acute changes occurring within a day or so of burning they are due to the direct effect of heat on erythrocytes The anæmia of burned patients nevertheless may increase in severity subsequently and may persist for many weeks (Brown 1944) The pathogenesis of the delayed anæmia of burning is clearly complex and in part at least it is due to dyshæmopoiesis the effects of protein loss and the presence *of necrotic tissue and sepsis* James Purnell and

Evans (1951) who carried out careful pigment excretion studies on burned patients concluded that increased hæmolysis was an important early factor particularly in patients with third-degree burns affecting more than 20% of the body surface

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CLINICAL FEATURES

Age and Sex The disease does not seem to be confined to any particular racial group and it has been observed in most countries of the world (Crosby 1953b) Both sexes are affected Most commonly the disease first appears in adult life usually in the third or fourth decade However a few cases in childhood have been recorded (Pierce and Aldrich 1943 Marks 1949) and elderly subjects also may be affected Paroxysmal nocturnal hæmoglobinuria has never been reported as being congenital and no instance of a familial incidence has been recorded Dameshek (quoted by Crosby 1953b) studied a patient who was one of identical twin sisters the other sister remaining healthy

Onset of the Disease The illness usually commences insidiously Hæmoglobinuria is often the first sign and characteristically this affects mainly or only urine passed during the night time or in the early morning on waking At the same time as the onset of hæmoglobinuria the patients usually complain of some of the symptoms such as weakness and dyspnœa on exertion which accompany chronic anæmia of moderate grade Some pallor is likely to be present and there may be mild jaundice

Course of the Disease In most patients the disease is a very chronic one It may not be severe and some patients are not seriously inconvenienced they become mildly to moderately anæmic with only occasional exacerbations at irregular intervals of weeks or even months in which occur attacks of nocturnal hæmoglobinuria lasting a few days Other less fortunate patients become severely anæmic and life becomes intolerable unless they are transfused they may have nocturnal hæmoglobinuria for months on end In the worst affected patients the hæmoglobinuria may be almost continuous lighter in the day time but seldom disappearing completely

In one of the author s patients (Case 32) hæmoglobinuria which appeared at intervals and lasted for a few days or a week or so was never demonstrably nocturnal The same was true of Bergmark s (1931) patient However this is unusual for the nocturnal rhythm is normally a characteristic and striking symptom Actually as will be discussed under *Pathogenesis* (p 420) the hæmoglobinuria is associated with sleep rather than with the night time the maximum amount of hæmoglobin being passed in the early hours of the morning (Abdicht Kuhlmann and Dencks 1942–43 Petersen 1949)

When the disease is active and hæmoglobinuria intense the

CHAPTER 16

PAROXYSMAL NOCTURNAL HÆMOGLOBINURIA

Synonyms Hæmolytic anæmia with perpetual hæmosideri nuria (Marchiafava 1928 Micheli 1928) splenomegalic anæmia with hæmoglobinuria hæmosiderinuria type Marchia fava Micheli (Donati 1930) chronic hemolytic anæmia with paroxysmal nocturnal hemoglobinuria (Hamburger and Bernstein *1936) Marchiafava Micheli* syndrome *(Scott Robb Smith and* Scowen 1938)

History The early reports in the literature on paroxysmal nocturnal hæmoglobinuria have been reviewed with unusual thoroughness by Crosby (1951) and it is now clear that the disease was recognized as a new entity far earlier than had been thought Strubing as long ago as 1882 differentiated the disease from other forms of hæmoglobinuria he realized that sleep was the determining factor in producing hæmoglobinuria and that neither cold nor exercise were immediate precipitating causes Strubing s detailed account and shrewd deductions were over looked or forgotten and the clinical features of the disease were only slowly pieced together in the next 50 years Paroxysmal nocturnal hæmoglobinuria (P N H) did not in fact become widely known until the 1930 s Now however it has an extensive literature and Crosby (1951) was able to find descriptions of at least 123 cases

With the exception of one publication little of importance in relation to the pathogenesis of the disease and the mechanism of hæmolysis had been recorded before the period 1935 to 1938 The exception was the report of Hijmans van den Bergh (1911) who showed that the disease was probably due to defective erythrocytes and that hæmolysis occurred *in vitro* in the presence of carbon dioxide About 25 years passed before these pioneer observations were confirmed and extended (Jordan 1935 1938 Ham 1937 Dacie Israels and Wilkinson 1938) Nevertheless despite a *greatly increased knowledge of the behaviour of* P N H erythro cytes *in vitro* and of the factors in normal plasma or serum which cause their hæmolysis the exact nature and the cause *of the* erythrocyte abnormality remain unsolved problems at the time of writing

reaction lying free or within casts or debris in the urinary deposit (see p 7) Hæmosiderinuria was remarked upon quite early in the description of the disease being recognized by Marchiafava and Nazari in 1911 Marchiafava (19 8) stressed its persistence when he referred to the disease as anemia emolitica con emosiderinuria perpetua

Quantitative Studies on Iron in Urine Relatively large amounts of iron are continuously lost in the urine in paroxysmal nocturnal hæmoglobinuria so much so that this may lead to iron deficiency in patients who have not been transfused

Cain and co-workers (1937) reported a daily loss of 6 5 to 8 5 mg of iron and Brulé Hillemand and Gaube (1938) a loss of 3 6 mg a day by a patient not suffering from hæmoglobinuria at the time of the examination

Analyses have been carried out by Dr D Marrack on three of the author s patients the average 24-hour excretions were 6 1 15 4 and 15 9 mg of iron

Atypical Forms of Paroxysmal Nocturnal Hæmoglobinuria

The description that has just been given of a chronic hæmolytic anæmia accompanied by bouts of hæmoglobinuria usually confined to the night time applies to the majority of cases of paroxysmal nocturnal hæmoglobinuria However patients may be seen in whom hæmoglobinuria occurs exceedingly infrequently and it seems likely that in the mildest cases hæmoglobinuria may be absent throughout the whole course of the disease In such cases the diagnosis may be far from obvious unless tests for the characteristic erythrocyte abnormality are deliberately carried out Dacie and Gilpin (1944) published an account of two brothers considered to be suffering from congenital aplastic anæmia (Fanconi s anæmia) The characteristic P N H erythrocyte abnormality was conclusively demonstrated in the elder brother despite the fact that he never had hæmoglobinuria (see p 445)

Rarely the true nature of the disease may be masked at least for a time by temporary or chronic marrow hypoplasia with resultant peripheral pancytopenia (Letman 1952 Nelson and Bruce 1953 Crosby 1953b and c)

In any instance therefore of obscure chronic hæmolytic anæmia or of apparent hypoplastic anæmia the remote possibility of the disease being paroxysmal nocturnal hæmoglobinuria should be borne in mind In some cases too the early symptoms of the disease may seem to have no relation to an anæmia of any sort Ellenhorn and co workers (1951) for instance described a patient whose presenting symptoms were recurrent attacks of nausea

patients develop a dusky reddish type of jaundice Siderosis may also contribute to a dusky complexion in patients who have received many transfusions

The episodes of hæmoglobinuria are not usually associated with any constitutional symptoms occasionally however the patient may complain of pain in the back Despite the continuous presence of hæmoglobinuria renal function is not seriously affected unless there is some unrelated complicating factor such as pyelonephritis Complaints of abdominal pain are not infrequent The spleen has been palpable in about half the recorded cases but it is seldom greatly enlarged Minor enlargement of the liver may also be found Venous thromboses develop not infrequently in the more seriously affected patients and produce a variety of signs and symptoms (Crosby 1953b) According to Crosby and Dameshek (1950) thrombosis is the commonest single cause of death in paroxysmal nocturnal hæmoglobinuria

Factors which Precipitate Attacks The attacks of hæmoglobinuria occur usually without apparent cause however a number of factors are known which may precipitate an attack Infections even quite minor ones will do this (Schally 1934–35 Ham 1939 Fisher 1947 Manchester 1945 Ross 1945) and in some patients menstruation acts in a similar way (Hitzenberger 1930 Fisher 1947) Both these factors have operated in one of the author s patients (Case 33) Transfusions also frequently provoke exacerbations of hæmolysis (Hamburger and Bernstein 1936 Ham 1939 Dacie and Firth 1943 Dacie 1948 Crosby and Stefanini 1952) and operations (including splenectomy) have the same effect

Taking iron salts by mouth often results in some unexplained way in an increase in hæmolysis (Strubing 1882, Iglauer and Frenreisz 1934 Cain Cattan Harrispe and van der Boijen 1937 Sega 1938 Hickey and Malley 1948) Hæmoglobinuria has also been provoked by injections of liver extract or T A B vaccine (Scott Robb Smith and Scowen 1938) and by other drugs given by injection or taken orally (Crosby 1953b)

Urine The hæmoglobinuria has already been mentioned the colour of the urine varies from pale red almost to black Albumin may be detected in the urine immediately before and after an episode of hæmoglobinuria (Crosby 1953b) but usually no protein can be demonstrated between the attacks of hæmoglobinuria A small increase in urobilinogen is however usual A constant finding is hæmosiderinuria i e the presence of iron containing granules giving the Prussian blue staining

are a macrocytic anæmia with the M C V increased up to 145 c μ a raised reticulocyte count a normal or slightly lowered hæmoglobin concentration a tendency to leucopenia largely due to neutropenia and slight to moderate thrombocytopenia

Stained blood films show macrocytes many of which stain diffusely basophilic sometimes slight diffuse punctate basophilia and a slight to moderate degree of anisocytosis and poikilocytosis A few normoblasts may be present in the most severely anæmic patients No spherocytes or evidence of erythrophagocytosis or abnormal leucocytes are usually seen Siderocytes and Heinz bodies are not found except after splenectomy The erythrocyte osmotic fragility is normal Relevant data obtained from six patients personally investigated are given in Table 28

The patients plasma (or serum) usually contains oxyhæmoglobin clearly visible to the naked eye if the disease is in an active phase In almost all patients the plasma will be found to have a slight to marked brownish tinge whether or not there is visible evidence of free hæmoglobin Such plasma will give a positive Schumm test for hæmatin and in most patients in whom hæmolysis is active the absorption bands of methæmalbumin can be seen with a spectroscope The bilirubin concentration does not commonly exceed 3 mg per 100 ml In mild cases or in quiescent phases it is often within the normal range in active phases it can be brought within the normal range by blood transfusion (Figs 97 and 98)

Pathology

Bone marrow The bone marrow is usually hypercellular due to increased erythropoiesis and the myeloid erythroid ratio may even be reversed Erythropoiesis is normoblastic or macronormoblastic (Fig 10 p 1C) but not megaloblastic Variable amounts of iron can be demonstrated by the Prussian blue reaction in erythrophages and normoblasts

The author has examined marrow films from four patients In one of them (Case 3) no iron could be demonstrated despite the fact that the patient had received more than 100 transfusions The local iron deficiency can probably be explained by loss of iron in the urine (see p 417) and by very active erythropoiesis leading to a rapid utilization of iron Iron was demonstrable in the marrows of the other three patients

The number of megakaryocytes in the marrow has been found to be diminished in certain patients with low peripheral platelet counts (Abdicht Kuhlmann and Dencks 1943) Scott Robb Smith and Scowen (1938) referred to three postmortem examinations in which

vomiting and abdominal pain The diagnosis of paroxysmal nocturnal hæmoglobinuria *was not made until the disease had* probably lasted for seven years during which time twenty two different diagnoses had been suggested !

Prognosis

Paroxysmal nocturnal hæmoglobinuria is essentially a chronic disease and many patients have lived for long periods after the diagnosis has been made Rosenthal (1932) recorded the history *of a patient who died after splenectomy 33 years after the onset* of symptoms and two patients studied by Stats Wasserman and Rosenthal (1948) survived 21 years Of the patients the writer has studied the disease has lasted for 8 years (Case 32) and 14 years (Case 33) respectively Both these patients are still alive and in fair health

According to Crosby (1953b) some patients show a tendency to improve as time passes the disease in two of Crosby s own patients having gradually become milder since the onset over ten years previously Crosby also mentioned that the patient described by Scheel (1925) did not subsequently suffer from hæmoglobinuria when examined in 1948 the patient was not anæmic (Hb 103%) although the leucocyte count (3 400 per *c mm) and the platelet count (118 000 per c mm)* were rather low

Case 34 of the author has apparently undergone a spontaneous cure after the disease had existed for at least 17 years the serological tests were negative in 1952 when he was last seen (see p 445) The patient described as Case 35 (A H of Dacie and Gilpin 1944) who was diagnosed in 1939 as suffering from congenital aplastic anæmia and paroxysmal nocturnal hæmoglobinuria has also recovered completely the serological tests which were still positive in 1942 being negative in 1948 and subsequently

Most patients however have died of a *combination of intercurrent* illness and their primary disease Marks (1949) gave the average duration of life of 21 patients as 6 6 years four deaths were directly attributable to the paroxysmal nocturnal hæmoglobinuria eleven died following operations (splenectomy in seven cases) and six patients died of unrelated disorders

Blood Picture in Paroxysmal Nocturnal Hæmoglobinuria

There are no characteristic features which enable a diagnosis of paroxysmal nocturnal hæmoglobinuria to be made from the blood count or by examination of a blood film The usual findings

megakaryocytes were reported to be scarce or absent and mentioned that megakaryocytes were scanty in one of their patients and present in normal numbers in the others

The author has examined bone marrow films from four patients from this point of view In one of them megakaryocytes appeared to be present in normal numbers in the others only a few could be found (see also *Pathogenesis* p 430) In the patient (Case F I Table 28) kindly referred by Professor L J Witts to the author biopsy revealed a marrow with some of the features of a hypoplastic anæmia fat cells were present although the patient was severely anæmic (Hb 4 0 g) there was a gross deficiency of leucocytes and megakaryocytes and there was an excess of plasma cells reticulum cells and mast cells This patient had a marked peripheral neutropenia and thrombocytopenia and a relatively low reticulocyte count Several other reports of acute or chronic marrow hypoplasia were mentioned by Crosby (1953b)

Spleen The size of the spleen is variable gross enlargement is rare According to Scott Robb Smith and Scowen (1938) the weights of eight spleens examined after removal at operation or autopsy ranged from 130 g to 720 g average weight 330 g (all the patients were adults) The histology of the spleen is not remarkable In particular little or no free iron can be demonstrated unless the patient has been heavily transfused during life nor is there evidence of excessive erythrophagocytosis

Liver Scott Robb Smith and Scowen (1938) reviewed the histology of the livers of ten patients The most generally reported finding was necrosis towards the centres of the lobules It was considered that this might have been due to impaction of erythrocyte stromata Tests for ionized iron gave negative results or at the most showed small amounts in Kupffer cells and liver parenchyma cells The livers were on the whole slightly enlarged the average of the recorded weights being 2 010 g

Kidneys The kidneys have usually been described as slightly enlarged and having conspicuously brownish red cortices Histologically the most remarkable feature is an intense siderosis of the convoluted tubules and of the loops of Henle Hæmosiderin containing debris may be seen in the collecting tubules As a rule there is no scarring or destruction of the nephrons (Crosby 1953b)

The marked degree of renal siderosis with little or no demonstrable iron in the rest of the organs of the body is the most characteristic feature of the pathology of paroxysmal nocturnal hæmoglobinuria It has been remarked on by many writers including Marchiafava and Nazari (1911) However it must be stressed that this remarkable restriction of iron to the kidney (due to the often continuous presence of hæmoglobin in the glomerular filtrate) is found only in patients who have not been transfused frequently In patients who have

TABLE 28 *Hæmatological data on six patients with paroxysmal nocturnal hæmoglobinuria*

Case No	Age	Sex	Erythrocytes millions (per mm.)	Hæmoglobin (g per 100 ml)	M.C.V. (c μ)	M C H C ( )	Leucocytes (per c mm)	Platelets (per c.mm)	Reticulocytes ( )	Bilirubin (mg per 100 ml.)
32	51	M	1 8	8 0	145	31	1 600–9 000	200 000–260 000	40–53	0 5–3 1
33	50	F	1 8	7 6	140	32	4 000–8 000	120 000–230 000	30–55	0 5–2 7
34	58	M	4 5–4 8	10 3–16 5	76–102	31 5–35	3 600	130 000	1 8–2 4	0 3–0 9
H M	35	F	2 3–3 5	8 0–11 1	100–138	28–32	2 600–6 000	240 000–300 000	2 2–17	0 3–0 8
A B	60	F	2 0–2 3	8 1–9 3	117–130	31–34 5	4 700–5 000		2 0–[illegible] 5	0 9–1 0
E L	22	F	1 1–1 3	4 0	89–104	35–38	1 000–2 100	2 000–13 000	4 5–7 0	0 4–0 6

depression of the respiratory centre during sleep might be the mechanism which activated hæmolysis

Ham (1937) showed that hæmolysis as judged by the degree of hæmoglobinæmia and hæmoglobinuria could be depressed if sufficient alkali were given and that acid given in the form of ammonium chloride caused an exacerbation of hæmolysis Ham (1939) demonstrated conclusively that the rhythm of hæmoglobinæmia and hæmoglobinuria could be reversed by making the patient sleep during the day time and stay awake at night under these circumstances the hæmoglobinuria occurred in the day time and not at night However the changes in blood *p*H that occur as the result of sleep are small and of themselves seem hardly sufficient to explain the striking increases in hæmolysis that may occur (Hoffman and Kracke 1943 McIlvanie and Beard 1951 Matthes Schubothe and Lindemann 1951) Crosby (1953b) moreover arranged for a patient to sleep in a Drinker respirator for ten nights The nocturnal rhythm of the hæmoglobinuria was unaltered even though the speed and amplitude of the respiratory cycle were adjusted so that they were greater than when the patient was awake thus ensuring that there was no possibility of retention of carbon dioxide

It is possible that other rhythmic changes occur e g in electrolyte concentration (Marks 1949) or even in the activity of certain of the clotting factors (Crosby and Dameshek 1950) or of complement (Rodbard 1950) and that a variety of slight changes occurring during sleep may have an important effect on the speed of the hæmolysis when acting in combination As will be shown later the plasma factors responsible for hæmolysis are complex hæmolytic factors and inhibitors seem to be so delicately balanced that very small changes in the activity of even one of the factors could have a marked effect on the hæmolytic activity of the plasma as a whole

The Erythrocyte Abnormality

The essential abnormality in paroxysmal nocturnal hæmoglobinuria is a defect in the erythrocytes the exact nature of which is still unknown That the patient s erythrocytes alone are defective is shown by the fact that whilst the patient s corpuscles are hæmolysed *in vitro* in normal serum as well as in his own serum normal corpuscles are ***not*** hæmolysed by the patient s serum *In vivo* too transfusion experiments lead to the same conclusion Normal corpuscles survive normally in patients with paroxysmal nocturnal hæmoglobinuria (Dacie and Firth 1943

received many transfusions siderosis will be marked in the liver spleen and other organs of the body despite the considerable amounts of iron that are excreted in the urine

Other Pathological Changes The only other possibly characteristic change is a predilection for thromboses (Scott Robb Smith and Scowen 1938 Ellenhorn *et al* 1951, Merliss 1952 Crosby 1953b) Crosby and Dameshek (1950) considered that defective platelets (see p 430) were perhaps an important factor in the genesis of thrombosis

PATHOGENESIS OF PAROXYSMAL NOCTURNAL HÆMOGLOBINURIA

The Nocturnal Hæmoglobinuria The constant presence of *hæmoglobinæmia the marked siderosis of the kidneys the* episodes of hæmoglobinuria and the ease with which hæmolysis takes place *in vitro* all indicate that a major part of the excess hæmolysis in paroxysmal nocturnal hæmoglobinuria takes place in the circulating blood stream It is likely that hæmolysis is continuous and that hæmoglobinuria occurs only when the rate of hæmolysis is increased to the point at which the amount of hæmoglobin in the glomerular filtrates exceeds the capacity of the renal tubules to re absorb the pigment (Yule 1942 Crosby and Dameshek 1950) There is reason to suppose that when hæmo*globinæmia is continuous the apparent renal threshold for excre*tion falls as the renal tubular cells become more and more saturated with hæmoglobin (Gilligan Altschule and Katersky 1941 Crosby 1953b)

As a rule the amount of hæmoglobin excreted in the urine in 24 hours is but a small proportion of the total amount catabolized Crosby (1953b) suggested that during periods of moderate activity only about 2% of the hæmoglobin broken down appeared in the urine With higher rates of hæmolysis the proportion would be considerably greater

The cause of the nocturnal rhythm of hæmoglobinuria is not yet clearly understood Strubing in 1882 pointed out that sleep was the determining factor and that neither exposure to cold nor exercise was important He also suggested that an accumulation of acid resulting from the previous day s activities might be the factor which provoked hæmolysis during sleep Ham (1937) and Dacie Israels and Wilkinson (1938) many years later knowing that *acidification in fact promoted hæmolysis in vitro* also suggested that accumulation of carbon dioxide as the result of

are suspended in fresh serum not all of them will be destroyed (Dacie 1948 Hickey and Malley 1948 Dacie and Mollison 1949) (Fig 94) Complete lysis has not been observed even with corpuscles from patients known to be severely affected In Cases 32 and 33 lysis has not exceeded 60 to 90% and in Case 34 (who was mildly affected) the proportion of erythrocytes lysed *in vitro* was approximately 40% in 1941 and 13% in 1947 when he was in remission Hickey and Malley (1948) in their patient recorded a figure of 50 to 60% of erythrocytes lysed and Wagley and Hickey (1948) 33% in another patient

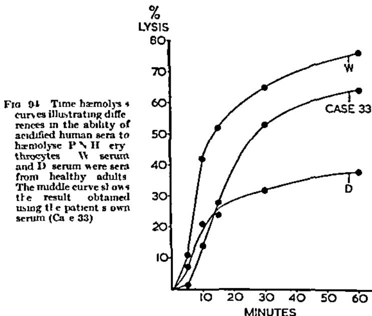


FIG 94 Time hæmolysis curves illustrating differences in the ability of acidified human sera to hæmolyse P N H erythrocytes W serum and D serum were sera from healthy adults The middle curve shows the result obtained using the patient s own serum (Case 33)

It seems likely therefore that a patient with paroxysmal nocturnal hæmoglobinuria forms erythrocytes which differ widely in their sensitivity to lysis some are so sensitive that they are almost immediately destroyed *in vitro* and have a very short life *in vivo* others are completely normal or so mildly affected that they are indistinguishable from normal A high proportion of the latter in the peripheral blood of a patient does not necessarily indicate that only a few abnormal erythrocytes are formed The more normal ones accumulate in relatively large numbers simply because their life span is much longer than the more affected corpuscles It should be added that reticulocytes and fully

Dacie 1948) (and if separated from the patient's erythrocytes by differential agglutination weeks after transfusion do not undergo lysis *in vitro* as do the patient's corpuscles) while patient's corpuscles are more or less rapidly destroyed after transfusion to normal recipients (Fig 93)

Not all the erythrocytes of a patient with paroxysmal nocturnal *hæmoglobinuria* are abnormally easily lysed *in vivo* and *in vitro*

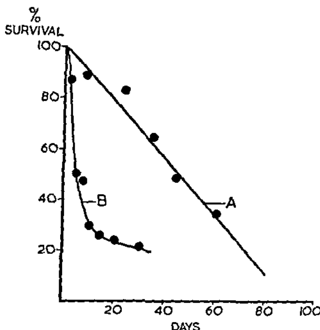


FIG 93 *The survival of normal erythrocytes in a patient suffering from paroxysmal nocturnal hæmoglobinuria (Case 33) A and of P N H erythrocytes (ex Case 32) in a normal adult recipient B (from Dacie and Mollison 1949)*

Dacie and Mollison (1949) transfused the blood of a patient to a healthy recipient and showed that after an initial period of rapid *destruction lasting 10 days or so during which time about 70% of the* transfused corpuscles were destroyed the rate of elimination was markedly slowed so that during the next 20 days only a further 10% of the cells were lysed (Fig 93) It seemed likely that the remaining corpuscles were surviving almost normally

In vitro studies also demonstrate differences in the sensitivity of P N H erythrocytes to lysis a few cells lyse within a minute or so the majority lyse within 5 to 20 minutes and a few undergo lysis in the next 20 to 40 minutes but even if the surviving intact corpuscles

that they also underwent lysis in an anti A serum more readily than did normal corpuscles

Dacie and Richardson (1943) reported further studies on the effect of pH *in vitro* and showed that if too much acid were added to the serum lysis might be inhibited (Fig 95 p 432)

Thus the main facts concerning the mechanism of hæmolysis *in vitro* in paroxysmal nocturnal hæmoglobinuria were established in Holland the United States and Britain in the years between 1935 and 1943 They have since been confirmed in many centres throughout the world and the use of acidified serum as an aid to the diagnosis of the disease has been widely referred to as Ham s (acid serum) test

Dacie (1949) reported that P N H erythrocytes if group A were regularly much more sensitive than were normal corpuscles to hæmolysis by the iso antibody anti A He found that the corpuscles of his group A patient (Case 33) were hæmolysed by anti A sera to approximately the same titre as they were agglutinated whereas normal group A corpuscles were agglutinated in anti A sera to far higher titres than they were hæmolysed and might not be hæmolysed at all P N H erythrocytes however were not hæmolysed in high titre anti D or anti M sera Dacie (1950) similarly observed that P N H erythrocytes were hæmolysed by cold antibodies to about the same serum dilutions as they were agglutinated (Table 29)

Hæmolysis due to anti A and cold antibodies is brought about by complement and the antibody and it seems probable that the abnormality of the P N H erythrocyte is one which facilitates the adsorption of complement with antibody to the cell surface thus converting an antibody the chief effect of which on normal corpuscles is agglutination into one which is actively hæmolytic

It is unlikely that a subtle abnormality at the erythrocyte surface would result necessarily in any characteristic morphological changes and this is borne out by the study of Romanowsky stained blood films or fresh blood viewed at ordinary magnifications However Matthes Schubothe and Lindemann (1951) have reported that electron micrographs of P N H corpuscles reveal an erythrocyte surface which is unusually pitted These most interesting observations need confirmation

Rodbard (1950) has argued that P N H erythrocytes probably have an abnormal lipoid surface structure He carried out experiments in an attempt to prove this hypothesis by exposing normal corpuscles to a variety of lipoid solvents In a small proportion of experiments by repeated washings in dilute solutions of alcohols at a relatively acid

ripened erythrocytes do not seem to differ in their sensitivity to lysis *in vitro* (Dacie 1948 Hickey and Malley 1948)

Hæmolysis of P N H Erythrocytes *in vitro*

In 1911 Hijmans van den Bergh showed that P N H erythrocytes underwent *hæmolysis in an atmosphere of carbon dioxide* but not in air Jordan (1935 1938) made further significant observations He concluded that the patient s corpuscles were already sensitized and that carbon dioxide caused hæmolysis by *potentiating the action of serum complement he also pointed* out that the coagulation of blood *in vitro* appeared to favour the development of hæmolysis

Ham s (1937) observations were also important He showed *that samples of whole clotted or defibrinated blood or blood* containing small amounts of heparin all underwent progressive hæmolysis when allowed to stand for 4 hours at 37 C or at room temperature and that exposure of heparinized or defibrinated blood to carbon dioxide caused *hæmolysis* to develop rapidly Ham also showed that lactic acid added to serum caused the serum to hæmolyse the patient s corpuscles and that the addition of sodium bicarbonate to serum decreased the hæmolytic action of carbon dioxide as did sodium citrate potassium oxalate and potassium cyanide Ham concluded that the onset of hæmolysis was related to *p*H but that reduction of *p*H in the absence of serum did not cause lysis He also showed that hæmolysis took place in normal serum as well as in the patient s serum that the serum factor essential for hæmolysis was destroyed by heating for 30 minutes at 50 or 60 C and that the activity of heated serum was not restored by the addition of fresh guinea pig serum

Ham s main observations were independently confirmed by Dacie Israels and Wilkinson (1938) In addition using hydrochloric acid as the acidifying agent they showed that the optimum *p*H for hæmolysis was in the region of *p*H 7 0 and that the lysin in the plasma was present in very low concentrations They observed that their patient s corpuscles underwent more lysis *in vitro* if the suspension of cells in acidified serum was chilled below 5 C before being warmed at 37 C than if the test was carried out at 37 C throughout

Further important observations were reported by Ham (1939) and Ham and Dingle (1939) Ham and Dingle observed that the erythrocytes of one of their patients were more sensitive to lysis by an anti human antibody than were normal corpuscles and

pH he was able to obtain corpuscles which were lysed in acidified serum in exactly the same way as were naturally-occurring P N H erythrocytes Unfortunately the results of these procedures were capricious and not easily repeatable Nevertheless there seems no reason to deny the possibility of the conversion *in vitro* of normal into P N H corpuscles particularly as enzymes such as trypsin have the property of profoundly altering the behaviour of erythrocytes and increasing their sensitivity towards certain antibodies However normal corpuscles when trypsinized behave very differently from P N H erythrocytes although there are some points of similarity (Table 29) A major difference is that trypsinized corpuscles are not hæmolysed in acidified normal serum whilst P.N H corpuscles are

Crosby (1953b) suggested that the stromal proteins of P.N H corpuscles were more likely to be abnormal than the lipoids of the cell surface or alternatively that the fundamental defect might be a deficiency in the enzyme systems which preserve and renew the stromal fabric of the erythrocytes This attractive hypothesis has yet to be proved

P N H erythrocytes almost always give negative direct antiglobulin tests i e there is no evidence that the cells have adsorbed abnormal globulins to their surface Nor is there evidence of any abnormal sensitivity to chemical hæmolysins (Ham and Dingle 1939 Dacie 1949) Actually positive antiglobulin tests have been recorded in at least one patient in a hæmolytic crisis (Caroli *et al* 1949) This was possibly due to the superimposition of auto immunization (see Crosby 1953b and p 434)

The Nature of the Plasma or Serum Factor

Jordan (1935 1938) drew attention to the possibility that the corpuscles of patients with paroxysmal nocturnal hæmoglobinuria were already sensitized and suggested that the serum factor might be complement he also stressed that the process of coagulation appeared to act as an activator of hæmolysis Ham (1937) and Dacie Israels and Wilkinson (1938) observed that the plasma or serum factor was thermolabile and that the activity of heated serum could not be restored by the addition of fresh guinea pig serum complement Ham and Dingle (1939) however concluded that the serum factor was closely associated with if not indistinguishable from complement or alexin of human serum Nevertheless they obtained some evidence which seemed to suggest that the P N H serum factor and complement were unlikely to be identical

Ham and Dingle showed that lyophilization of normal serum reduced its hæmolytic activity against P N H erythrocytes without altering its complement activity and that heating from 45 to 50 C filtration

TABLE 29 *Comparison of the sensitivity to agglutination and hæmolysis of P N H erythrocytes normal erythrocytes and trypsinized normal erythrocytes*

Erythrocytes	Type of antibody or serum													
	Anti A		Cold antibody (Case 14)		Anti M		Anti D (In saline)		Anti D (In serum albumin)		Donath Landsteiner antibody	Warm lysin (Case 13)	Reversible lytic factor	Normal serum (pH 6 5 7 0)
	A (0° C)	H (37° C)	A (20° C)	H (0° C)	A (20° C)	H (37° C)	A (37° C)	H (37° C)	A (37° C)	H (37° C)	H (0° C–37° C)	H (37° C)	H (37° C)	H (37° C)
P N H (Case 32)			4 096	4 096			0	0	1 024	0	32	512	0	+++
P N H (Case 33)	256	256	4 096	2 048	64	0	0	0	1 024	0	32			+++
P N H (H M)	256	128	4 096	2 048							16			+
Normal	128	0	4 096	0	64	0	0	0	512	0	8	0	0	0
Trypsinized Normal	512	4	16 000	256			512	0			16	128	16	0

The figures refer to agglutinin or hæmolysin titres A = agglutination H = hæmolysis = no observation

* This hæmolytic factor active against trypsinized cells only was described by Hurley and Dacie (1953)

Lyons (19[illegible]) confirmed the activating action of thrombin He claimed moreover that P N H erythrocytes would undergo hæmolysis in any isotonic medium in which a fibrin clot was produced and that lysis would take place even when erythrocytes were suspended in fibrinogen solutions subsequently clotted by thrombin Crosby and Dameshek's (1950) observation were also confirmed by Martin and Ross (1953)

The identity of the hæmolytic factor with Ac globulin was not accepted by Harris Jordan Pillemer and Desforges (1951) who found that serum dialysed against normal saline lost its hæmolytic activity against P N H cells but retained its complement and Ac globulin Hæmolytic activity was nevertheless restored after adding a magnesium salt Again treatment of serum with barium sulphate permanently reduced Ac globulin activity but P N H activity was regained on adding magnesium They also found that the removal of any of the components of complement from serum resulted in the loss of hæmolytic activity against P N H cells They deduced from these and other experiments that the serum factor resembled a metal requiring enzyme probably a proteinase which differed from human complement by its dependence on magnesium and its inhibition by calcium

Clapp Williams and Mendel (1952) brought forward evidence of the interaction of at least two factors One of these factors was heat labile magnesium dependent and fluoride inhibited and probably *identical with the factor of Harris and co workers (1951) referred to in* the preceding paragraph the other was an adsorbable globulin which was probably heat stable They concluded from the reactions of the heat labile and heat stable fractions that it was still possible that they were fractions of complement

Crosby (1953a and b) in his latest papers has postulated that *four* distinct factors are involved all probably proteins Two of the factors are hæmolytic against P N H erythrocytes but not against normal corpuscles the other two factors inhibit hæmolysis One of the hæmolytic factors is heat labile and the other heat stable similarly of the two inhibitors one is heat labile and the other heat stable Both calcium and magnesium are necessary for hæmolysis but in excess both cause inhibition Thrombin destroys the heat stable inhibitor rapidly and the heat labile hæmolytic factor slowly it acts therefore as an activator of hæmolysis Heparin and protamine in appropriate dosages increase hæmolysis by blocking the inhibitors

The work described in the preceding paragraphs indicates that the serum factor active against P N H erythrocytes is a complex of several substances Whether these factors which may be enzymes take part in normal physiological reactions remains to be seen their existence in normal serum probably indicates they have some normal function Judging from Crosby s work they seem to form a delicately poised equilibrium of activators

through Berkfeld candles and storage at room temperature removed hæmolytic activity more readily than complement activity They could not demonstrate any adsorption of complement by P N H erythrocytes in excess of that adsorbed by normal corpuscles when acidified serum was subjected to successive absorptions with normal or P N H corpuscles respectively They found nevertheless that the addition of fresh guinea pig serum restored hæmolytic activity to zymin treated or ammonium hydroxide treated human serum (lacking the C 3 and C 4 fractions of complement respectively)

Dacie Israels and Wilkinson (1938) and Ham and Dingle (1939) observed that the amount of lysis produced by a human serum was significantly increased by the addition of fresh guinea pig serum Dacie (1949) showed however that much of this increased hæmolysis was caused by the presence in the guinea pig serum of anti human antibodies to the lytic effect of which P N H erythrocytes are unusually sensitive Dacie (1949) found that there was a positive correlation between the hæmolytic power of a series of normal human sera against P N H erythrocytes and the complement content of the sera as judged by their ability to hæmolyse sensitized sheep cells He pointed out however that this did not prove that the factors concerned were identical and stressed that the range of *p*H within which hæmolysis of P N H erythrocytes occurred was significantly narrower than the *p*H range for the lysis by human complement of corpuscles sensitized by known amboceptors

Wagley and Hickey (1948) also brought forward evidence against the identity of complement and the P N H lytic factor They found that the addition of acidified serum previously heated at 56 C to fresh acidified serum reduced the hæmolytic activity against P N H erythrocytes more than it reduced complement activity and that acidification to *p*H 5 1 destroyed hæmolytic activity against P N H corpuscles without destroying complement when the sera were subsequently tested after readjusting their *p*H to 6 4

More recent work has underlined the complexity of the problem Crosby and Dameshek (1950) observed that the hæmolytic activity of normal serum was increased by the addition of thrombin and Crosby (1950) suggested that the activating effect of thrombin on the hæmolytic system might be used as a specific test for paroxysmal nocturnal hæmoglobinuria Crosby and Dameshek concluded that the hæmolytic factor existed in plasma as an inert precursor which was activated by the process of coagulation into an active hæmolytic agent They suggested that the heat labile hæmolytic factor was similar to if not identical with the serum coagulation accelerator (Ac globulin or Factor VI)

absent In these patients even a careful history is very little help in diagnosis

In other patients the history may be misinterpreted hæmoglobinuria may be assumed to be hæmaturia and the patient investigated accordingly or the significance of its periodicity may not be appreciated One of the author s patients (Case 33) told her physician that she passed dark urine When she was asked to pass a sample for testing (in the day time) it was found to be perfectly normal and her story was therefore discounted She was thereupon treated quite fruitlessly for pernicious anæmia! It is in fact only in recent years that paroxysmal nocturnal hæmoglobinuria has been diagnosed with any frequency and promptness

Whilst the presence of hæmoglobinæmia hæmoglobinuria and hæmosiderinuria and the signs of compensatory increased erythropoiesis all point to intravascular hæmolysis and hæmolytic anæmia a positive diagnosis of paroxysmal nocturnal hæmoglobinuria cannot be made except by the demonstration of the characteristic erythrocyte abnormality

The Acid serum Test

This test can be carried out with the patient s corpuscles and serum or with the patient s corpuscles and normal compatible serum The patient s serum is best obtained by defibrination as serum expressed from clotted blood is usually far more hæmolysed (The exact technique is described on p 498) The amount of acid to be added for maximal hæmolysis depends to some extent on the serum and also on the proportion of patient s corpuscles subsequently added Ham (1939) recommended 5% by volume of 0 85N lactic acid or N/3 HCl the author adds 10% by volume of either N/5 or N/4 HCl and then a one tenth volume of a 50% saline suspension of washed patient s erythrocytes Lysis is almost maximal after 30 minutes at 37 C (Fig 94) It is often more convenient to use compatible normal serum instead of the patient s serum and as sera vary in their ability to cause the lysis of P N H erythrocytes (Dacie and Mollison 1949) it is worth while to choose a normal serum known to be potent in this respect Representative *p*H hæmolysis curves are illustrated in Fig 95 the inhibition due to over acidification is best shown with the less active serum

The specificity of the acid serum test was considered by Dacie (1949) If carried out carefully with adequate controls a positive test i e lysis in the acidified serum but little or no lysis in unacidified serum is diagnostic of the P N H abnormality The

and inhibitors whose resultant activity is easily disturbed It is probable that the sensitivity of the hæmolytic system is the reason why the clinical pattern of hæmolysis is so easily affected by subtle changes associated with sleep and less commonly but sometimes more drastically activated by intercurrent illness operations and transfusions

The Leucopenia and Thrombocytopenia

The frequency of thrombocytopenia and leucopenia in paroxysmal nocturnal hæmoglobinuria has been referred to on p 417 The exact cause is obscure Crosby (1953b) reported that the leucocytes and platelets of a patient with paroxysmal nocturnal hæmoglobinuria underwent autolysis *in vitro* more rapidly than did the leucocytes and platelets of normal subjects He suggested that they suffered from some defect analogous to the defect in the P N H erythrocyte In other rare cases thrombocytopenia and leucopenia (and reticulocytopenia) may be so severe as to suggest that the patient is suffering from aplastic or ' refractory anæmia (see p 415 and Table 28) In these patients the peripheral cytopenia appears to be at least in part a reflection of marrow hypoplasia It is uncertain whether this should be looked upon as an exaggeration of the P N H defect producing cell destruction in the marrow or inhibition of hæmopoiesis or as the simultaneous presence of idiopathic marrow aplasia as perhaps in Dacie and Gilpin s (1944) case The pancytopenia is essentially of relatively long duration it seems to be distinct from the transient acute episodes most of which are probably due to infections which occur in the course of other types of hæmolytic anæmia as well as in paroxysmal nocturnal hæmoglobinuria (Crosby 1953b and c) (see p 16)

DIAGNOSIS

Paroxysmal nocturnal hæmoglobinuria can often be diagnosed tentatively from the patient s description of his or her symptoms A history in an adult of repeated episodes of passing dark urine with weakness pallor and jaundice extending over a matter of months or even years is very suggestive and if the patient says that the dark urine is passed particularly on waking in the morning the diagnosis of paroxysmal nocturnal hæmoglobinuria is almost certain Nevertheless as already mentioned anæmia and jaundice may be mild and hæmoglobinuria infrequent or perhaps even

corpuscles in acidified sera Crosby (1950) showed that more lysis of P N H erythrocytes was produced by an acidified serum if thrombin was added to the cell serum suspension and proposed that this might be used as a specific test for the disease

Another characteristic finding in paroxysmal nocturnal hæmoglobinuria is the rapidity with which spontaneous lysis occurs when samples of patient s clotted blood or blood defibrinated under paraffin or in a closed vessel or lightly heparinized blood are allowed to stand at room temperature or at 37 C (Ham 1937 Dacie Israels and Wilkinson 1938) Hegglin and Maier (1944) suggested that the heat resistance of erythrocytes i e the development of hæmolysis when clotted blood was incubated at 37 C might be used as a specific test for paroxysmal nocturnal hæmoglobinuria This is certainly a striking characteristic of the blood of patients with paroxysmal nocturnal hæmoglobinuria but it is not diagnostic of the disease The author has observed equally rapid lysis in acquired hæmolytic anæmia with very marked spherocytosis (e g Case 12) and in hæmolytic anæmia due to cold hæmolysins of high thermal amplitude (e g Case 18) unless in the last instance the strictest precautions were taken to prevent the blood becoming chilled to room temperature after withdrawal Moreover in paroxysmal nocturnal hæmoglobinuria the heat resistance test may fail or be inconclusive it may fail if the clot does not contract satisfactorily (due to thrombocytopenia) and it may be inconclusive if for some other reason the clot fails to retract spontaneously and has to be manipulated

Finally the results of certain other tests have some diagnostic importance As mentioned on p 427 the direct antiglobulin test is negative in uncomplicated cases of paroxysmal nocturnal hæmoglobinuria Tests for abnormal antibodies in the patients sera are likewise typically negative although as the patients often receive repeated transfusions immune iso antibodies are sometimes formed (see Case 33 p 444)

Atypical Laboratory Findings in Paroxysmal Nocturnal Hæmoglobinuria

The characteristic erythrocyte abnormality and the leucopenia and thrombocytopenia are not the only abnormal hæmatological findings which have been observed in patients suffering from paroxysmal nocturnal hæmoglobinuria Erythrocyte osmotic fragility for instance was reported by Abdicht Kuhlmann and Dencks (1942–43) to be markedly increased It is possible that this was due to the superimposition of paroxysmal nocturnal

really essential control is a suspension of normal erythrocytes in a duplicate sample of the acidified serum—the normal corpuscles must not undergo hæmolysis An additional control to be set up if the patient's corpuscles are markedly spherocytic (which in itself is strongly against the diagnosis of paroxysmal nocturnal hæmoglobinuria) is a suspension of patient's corpuscles in acidified normal serum previously inactivated at 56 C If lysis occurs in the acidified heated serum as well as in acidified unheated serum, this is probably due to the spherocytosis and not to the P N H erythrocyte abnormality

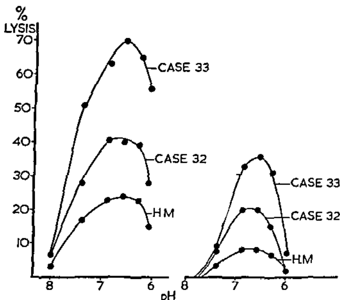


FIG 95 The effect of *p*H on the hæmolysis *in vitro* of P N H erythrocytes by normal human sera Erythrocytes from three different patients were used (Cases 32 and 33 and H M) and two different normal adult sera (W serum on the left and D serum on the right)

Other Tests for Paroxysmal Nocturnal Hæmoglobinuria

Other confirmatory tests can be carried out The greatly increased sensitivity of P N H corpuscles to hæmolysis by high titre cold antibodies or in the case of group A patients to hæmolysis by anti A can be used in diagnosis

These reactions seem to be as characteristic of paroxysmal nocturnal hæmoglobinuria and at least as sensitive in differentiating the abnormal from the normal as lysis of the patients

Splenectomy has been undertaken on many occasions the majority of patients have failed to benefit and the mortality from the operation has been high

The Administration of Alkalies

Following the observation that hæmolysis *in vitro* was increased by a reduction in *p*H it was logical to attempt to inhibit hæmolysis *in vivo* by the administration of alkalies The results of such treatment however were unsatisfactory

Ham (1939) found that sodium bicarbonate given orally in large doses (65 g in 24 hours) produced a transient decrease in the hæmoglobin content of the plasma and urine of two patients One patient was treated continuously with large doses of alkaline salts (40 g of sodium bicarbonate daily for 13 days and 56 g of sodium citrate daily for 3 days) hæmoglobinuria was absent during the first eight days of this treatment but thereafter it was present at night time although the treatment was being continued In both patients intense hæmoglobinuria lasting day and night followed the sudden cessation of the alkali therapy Similar unsatisfactory results were reported by Buell and Mettier (1941)

The exacerbations of hæmolysis recorded by Ham when the alkali therapy was stopped were presumably due to the accumulation of very sensitive cells in the circulation while hæmolysis was depressed When conditions were again favourable for hæmolysis large numbers of sensitive cells would be destroyed within a short time This phenomenon is likely to be encountered in any treatment which depresses the activity of the patient s plasma without reducing the sensitivity of his cells

Dicoumarol

Crosby and Dameshek (1950) described the effect of dicoumarol in one patient They observed that when the serum accelerator activity was depressed to 30% of the normal the plasma hæmoglobin concentration fell and hæmoglobinuria ceased In order to achieve this the plasma prothrombin activity had to be reduced to potentially dangerous levels Moreover dicoumarol did not prevent the activation of the disease which followed a respiratory infection nor a hæmolytic episode provoked by transfusion When the dicoumarol was discontinued hæmolysis increased in intensity Dicoumarol is thus disappointing as a therapeutic agent in paroxysmal nocturnal hæmoglobinuria It has however some value and seems worthy of trial in patients subject to thromboses (Crosby 1953b)

Heparin *In vitro* heparin inhibits hæmolysis if present in sufficient concentration (Ham 1939 Dacie and Richardson 1943) very low concentrations enhance hæmolysis (Crosby and

hæmoglobinuria on a pre existing hereditary spherocytosis Alternatively and perhaps more likely the increased osmotic fragility was caused by the simultaneous formation of auto antibodies as may have happened in the patient of Caroli and co workers (1949)

It is possible but yet hardly proved that the clinical syndrome of paroxysmal nocturnal hæmoglobinuria may be brought about by a second and quite distinct hæmolytic mechanism At least two patients have been described whose paroxysmal nocturnal hæmoglobinuria may have been due not to an erythrocyte abnormality but to a hæmolysin in the patient s serum (Enneking 1928 Heilmeyer and Wengeler, 1943)

The exact significance of these observations is uncertain at the present time In Heilmeyer s and Wengeler s case the hæmolysin was shown to be most active at a pH of 6 0 to 6 5 It should be added that Schubothe (1953 personal communication) could not demonstrate any abnormal antibodies when he re ently reinvestigated this patient Possibly auto immunization had been superimposed on true paroxysmal nocturnal hæmoglobinuria in this case as also perhaps in the patient of Hollander Ludwig Siemsen and Walser (1953)

Liu (1951) described two patients whom he considered might be suffering from an atypical form of paroxysmal nocturnal hæmoglobinuria or a hitherto undescribed type of chronic hæmolytic anæmia with erythrocyte fragility to cold and acid In one patient hæmoglobinuria followed exposure to cold and in the other it followed one of several blood transfusions It never occurred spontaneously However intravascular hæmolysis was constantly present as shown by raised plasma hæmoglobin concentrations no nocturnal increase in plasma hæmoglobin was demonstrated

In retrospect it seems probable that Liu s patients were in fact suffering from paroxysmal nocturnal hæmoglobinuria The behaviour of their erythrocytes as described by Liu is compatible with that diagnosis As observed by Dacie Israels and Wilkinson (1938) chilling may increase the amount of lysis ob ervable *in vitro* because of the great sensitivity of P N H corpuscles to the potentially hæmolytic cold antibodies present in low concentration in many normal human sera (Dacie 1950)

TREATMENT

No specific treatment is possible as the cause of the essential defect of the P N H erythrocyte is unknown Nevertheless attempts have been made to reduce the activity of the patients plasma factors by the administration of alkalies dicoumarol A C T H and other drugs These measures however give at the best only transient relief Blood transfusions on the other hand are of real value although their effects are only temporary

patients died shortly after the operation Death seems to have resulted from a variety of causes shock ' hæmorrhage exacerbations of hæmolysis and postoperative thromboses being contributory factors Nevertheless a few patients seem to have derived some benefit from the operation the nocturnal hæmoglobinuria becoming less frequent (Dacie Israels and Wilkinson 1938 Ham 1939 Ham and Horack 1941 Fisher 1947 Barnett Dunlop and Pullar 1951 Andersson 1952) However it is almost impossible to be certain that any improvement was really due to removal of the spleen as the course of the disease is so variable

There seems to be no reason to recommend splenectomy The benefits if any are so small that they cannot be held to outweigh the risks of the operation even allowing for the fact that the immediate mortality from the operation would nowadays be far smaller than formerly

Transfusion

Blood transfusion is undoubtedly the most beneficial form of treatment for paroxysmal nocturnal hæmoglobinuria at present available The survival of normal erythrocytes is usually unimpaired and this makes transfusion of seriously anæmic patients well worth while However some patients are relatively intolerant of transfusion and regularly experience major or minor exacerbations of hæmolysis as well as rigors and pyrexia following transfusion of apparently compatible blood (Hamburger and Bernstein 1936 Ham 1939 Ross 1945 etc) Nevertheless satisfactory rises in hæmoglobin and remissions from hæmoglobinuria have been reported following transfusions even in patients who react badly to them For instance in Ham s first patient a severe reaction was followed by two weeks of freedom from hæmoglobinuria and only slight nocturnal amounts occurred in the succeeding two weeks

Dacie and Firth (1943) studied in detail the effect of transfusing a patient (Case 33) with 500 ml of a concentrated suspension of group O blood (unwashed) A very severe hæmolytic reaction followed from which the patient nevertheless made a good recovery Almost black urine was passed during the 48 hours following the transfusion The urine subsequently remained free from hæmoglobin for about six weeks Within 24 hours of the commencement of the transfusion the patient became strikingly brownish yellow in colour the brownish tinge disappeared within a few days but the jaundice persisted longer although eventually it cleared almost completely The total erythrocyte count of the patient gradually rose following the transfusion reaching its highest point five weeks later (Fig 96) Fortunately the

Dameshek 1950) Crosby and Dameshek reported that a patient with paroxysmal nocturnal hæmoglobinuria to whom 8 000 units of heparin were given in the course of a blood transfusion developed a severe hæmolytic reaction Although the heparin was not proved to be the cause of the reaction Crosby and Dameshek concluded that it was probably dangerous to use heparin *in vivo* in view of its activating power in small concentrations on hæmolysis *in vitro* More recently Nelson and Bruce (1953) reported another severe hæmolytic reaction following the administration of heparin

α Tocopherol Phosphate α Tocopherol phosphate acts as an anti coagulant probably by serving as an antithrombin Williams and Clapp (1953) administered the drug to a patient with paroxysmal nocturnal hæmoglobinuria in the hope that it would inhibit the plasma hæmolytic factor However despite apparent inhibition of the acid serum test *in vitro* the patient derived no benefit The authors concluded that the drug had no therapeutic value

Parasympathomimetic Drugs Hoffman and Kracke (1943) treated a patient with prostigmine eserine and pilocarpine respectively Hæmoglobinuria was temporarily abolished by each drug but the erythrocyte count was hardly altered pilocarpine appeared to be the most effective Marks (1949) also used pilocarpine and noted clearing of hæmoglobinuria but the drug had to be discontinued because of side effects Simpson and Oldham (1950) found pilocarpine to be of no value McIlvanie and Beard (1951) administered prostigmine to one patient and pilocarpine to two but they derived no decisive benefit

A C T H and Cortisone

A C T H and/or cortisone have been tried on several occasions but without significant benefit Essentially negative results were reported by Kalant and Cyr (1952) and Hoffman and Powell (1952) in America by the M R C Hæmatology Panel (1952) in two cases in England by Nelson and Bruce (1953) and others (see Crosby 1953b) The abdominal pain of the patient of Fudenberg Palmer and Kirsner (1954) however was alleviated

Adrenalin was used by Hoffman and Kracke (1943) but without benefit According to Crosby (1953b) it can bring about short lived suppression of hæmolysis

Splenectomy

Splenectomy was frequently undertaken in cases of paroxysmal nocturnal hæmoglobinuria at a time when little or nothing was known regarding the hæmolytic mechanism of the disease The results of the operation were generally disappointing and the mortality high according to Crosby (1953b) eight out of 34

Stefanini believed that the hæmoclastic reaction was followed by increased hæmolysis only in paroxysmal nocturnal hæmoglobinuria the hæmolysis becoming obvious several hours after the transfusion at about the time the pyrexia subsided and sometimes continuing at a rapid rate for several days

Indications for Transfusion

Transfusion needs to be used with discretion as a palliative treatment for paroxysmal nocturnal hæmoglobinuria If the patient is chronically severely anæmic with a hæmoglobin concentration persistently below 7 g per 100 ml he certainly should not be denied the very considerable benefits from transfusion even if this means commencing a transfusion life and subjecting him to the risk of transfusion reactions and the more remote risk of serum hepatitis Less severely anæmic patients with hæmoglobin concentrations averaging about 10 g per 100 ml should not be transfused for they become well adapted to their anæmia and usually recover rapidly from any more serious hæmolytic episodes

The two patients described as Cases 32 and 33 each become seriously anæmic unless transfused Both have been transfused with washed erythrocytes at intervals from 1947 up till the time of writing This has been carried out usually at eight week intervals the patients receiving as a rule the washed cells from four to six pints of blood divided into two or three transfusions at daily or two day intervals The transfusions have almost always been received without rises in temperature or significant symptoms or exacerbations of hæmolysis

The patients have usually been able to lead active lives for about six weeks or so following the transfusions sometimes also being free from hæmoglobinuria The possibly more desirable plan of transfusing them as outpatients at say weekly intervals with the washed corpuscles from one pint of blood so as to maintain a steadier hæmoglobin concentration has not been carried out because of the distance the patients live from hospital

Especial care must be taken with cross matching tests because of the possibility of the formation of immune iso antibodies Case 33 developed anti *Kell* and this was the cause of a severe reaction before it was discovered Case 32 on the other hand has not yet developed any iso antibodies The other problem to be considered is that of post transfusion siderosis The tissues of both patients by now probably contain a great deal of iron The excess iron however does not seem to be doing ha[rm] although no doubt undesirable this has not been cons[idered]

other workers and there seems no doubt that the method is of value in patients who are sensitive to whole blood transfusions (Caroli *et al* 1949 Bousset and Vernant 1949 Dameshek and Neber 1950 Schubothe and Matthes 1951 Crosby 1953b)

It is not claimed that all patients who suffer from paroxysmal nocturnal hæmoglobinuria and need transfusion require to be transfused with washed erythrocytes some certainly seem quite tolerant of whole blood

The Plasma Transfusion Reaction The cause of the hæmolytic reactions which sometimes follow transfusions given to patients with paroxysmal nocturnal hæmoglobinuria requires further discussion Dacie and Firth (1943) and Dacie (1948) attributed the severe hæmolytic reactions that they observed to the transfusion of potentially hæmolytic plasma components which it was assumed were in short supply in the patient s own plasma The very severe hæmolytic reaction experienced by the patient described as Case 33 who was group A was undoubtedly due mostly to the transfusion with the unwashed packed group O corpuscles of small amounts of anti A to which P N H corpuscles are extremely sensitive and by which they are rapidly hæmolysed (see p 425) The hæmolytic reactions to unwashed corpuscles experienced by the patient described as Case 32 were much less severe He was group O and received compatible group O blood the harmful components in the transfused plasma were not identified

Dameshek and Neber (1950) described the occurrence in certain susceptible patients of a transfusion reaction which could be avoided by the transfusion of washed corpuscles Chill pyrexia backache and pain in the legs were common symptoms but there were no signs that the reaction was accompanied by hæmolysis except in paroxysmal nocturnal hæmoglobinuria The reaction was encountered in patients suffering from a variety of diseases Most of them had been transfused previously on many occasions

Crosby and Stefanini (1952) and Crosby (1953b) have studied the plasma transfusion reaction in more detail They considered that the reaction regularly followed transfusion in paroxysmal nocturnal hæmoglobinuria and that a hæmolytic crisis was an invariable sequel to it They concluded that a heat labile factor of unknown origin was responsible and that certain objective changes followed in a definite sequence e g leucopenia and thrombocytopenia and an increase in the coagulability and fibrinolytic activity of the blood occur at the time of the chill and commencement of pyrexia They pointed out that the pattern of changes was similar to that produced by anaphylactic and peptone shock by incompatible transfusions and by the injection of foreign proteins such as T A B vaccine (known to cause hæmolytic episodes in paroxysmal nocturnal hæmoglobinuria) Crosby and

Stefanini believed that the hæmoclastic reaction was followed by increased hæmolysis only in paroxysmal nocturnal hæmoglobinuria the hæmolysis becoming obvious several hours after the transfusion at about the time the pyrexia subsided and sometimes continuing at a rapid rate for several days

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became con picuous Paroxysmal nocturnal hæmoglobinuria was diagnosed in 194

Physical Examination (1942) She was a well nourished woman of medium height and weight pale and just perceptibly jaundiced The tip of the spleen was just palpable The urine passed between the early hours of the morning and 9 a m contained hæmoglobin it was

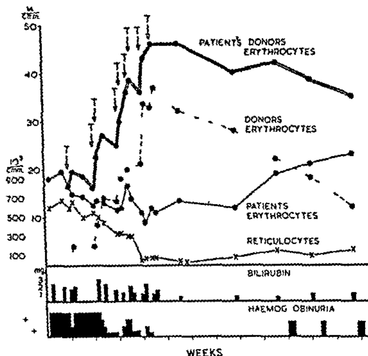


FIG 98 Hæmatological observations on a patient with paroxysmal nocturnal hæmoglobinuria (Case 33) as the result of a series of transfusions of saline washed normal erythrocytes (redrawn from Dacie 1949) T = transfusion The reticulocytes are recorded in absolute numbers (× 10³ per c mm)

normal in colour at other times Hæmosiderin was detected in the urinary deposit

Laboratory Findings (1942) There were 2 200 000 erythrocytes per c mm and 7 g of hæmoglobin per 100 ml the M C V was 113 c μ and there were 10% reticulocytes The total leucocyte count was 5 500 per c mm with 50% neutrophils and the serum bilirubin concentration 1 4 mg per 100 ml Stained blood films showed slight anisocytosis and macrocytosis and polychromasia The erythrocyte osmotic fragility was normal Bone marrow biopsy revealed active erythroblastic

absolute contraindication to continuing the transfusions Fortunately both patients continuously excrete a relatively large amount of iron in their urine (p 415) and this to some extent counterbalances the effect of so many transfusions

Case Report Paroxysmal Nocturnal Hæmoglobinuria

Case 32 The patient (H A) is a man now aged 51 years Hæmoglobinuria was first noticed in September 1946 but he had been unwell for six months before this Hæmoglobinuria reappeared in January 1947 and paroxysmal nocturnal hæmoglobinuria was diagnosed His earlier history was described by Dacie (1948)

Physical Examination (1947) He was a slightly jaundiced pale thin man The spleen was just palpable but there were no other abnormal physical signs His urine contained hæmoglobin and there was a large amount of hæmosiderin in the urinary deposit

Laboratory Findings (1947) There were 1 800 000 erythrocytes per c mm and 8 0 g of hæmoglobin per 100 ml the M C V was 145 c μ and there were 40 to 53% reticulocytes The leucocyte count averaged 3 800 cells per c mm and the platelet count averaged 230 000 per c mm (see also Table 28) Stained blood films showed a moderate degree of anisocytosis many macrocytes and a few poikilocytes His bone marrow was very hyperplastic erythropoietic cells predominating (Fig 10 p 16)

The acid serum test was positive the antiglobulin test negative and erythrocyte osmotic fragility normal

Further Progress In August 1947 he received a series of transfusions of saline washed erythrocytes The erythrocyte count was temporarily restored to normal and his hæmoglobinuria and jaundice disappeared for a time (Fig 97) Since then he has been transfused with washed cells at intervals of about eight weeks as described on p 441 and has been maintained in this way in fair health His disease however shows no signs of any real diminution in its intensity

The clinical story of this patient is remarkable in that the hæmoglobinuria has never been obviously nocturnal As a rule attacks of hæmoglobinuria have occurred at intervals of weeks or months and have lasted usually for several days on end without apparent intermission On the whole he has experienced much less hæmoglobinuria than the patient next described (Case 33) who is suffering from paroxysmal nocturnal hæmoglobinuria of about the same degree of severity as judged by the severity of her anæmia and the reticulocyte response

Summary A case of severe paroxysmal nocturnal hæmoglobinuria of eight years duration Hæmoglobinuria has never been obviously nocturnal The patient has been maintained in fair health by means of a series of transfusions of saline washed erythrocytes

Case Report Paroxysmal Nocturnal Hæmoglobinuria

Case 33 The patient (K L) is a single woman now aged 50 years Her earlier history was reported by Dacie and Firth (1943) and Dacie (1948) Her illness dates from November 1940 Hæmoglobinuria at night was the first sign of the disorder and later pallor and jaundice

became conspicuous Paroxysmal nocturnal hæmoglobinuria was diagnosed in 194_.

Physical Examination (1942) She was a well nourished woman of medium height and weight pale and just perceptibly jaundiced The tip of the spleen was just palpable The urine passed between the early hours of the morning and 9 a m contained hæmoglobin It was

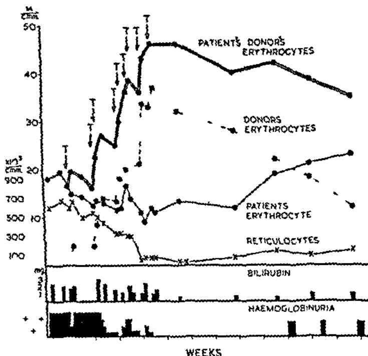


FIG 98 Hæmatological observations on a patient with paroxysmal nocturnal hæmoglobinuria (Case 33) as the result of a series of transfusions of saline washed normal erythrocytes (redrawn from Dacie 1949) T = transfusion The reticulocytes are recorded in absolute numbers (× 10³ per c mm)

normal in colour at other times Hæmosiderin was detected in the urinary deposit

Laboratory Findings (1947) There were 2 200 000 erythrocytes per c mm and 7 g of hæmoglobin per 100 ml the M C V was 113 c μ and there were 10% reticulocytes The total leucocyte count was 5 500 per c mm with 50% neutrophils and the serum bilirubin concentration 1.4 mg per 100 ml Stained blood films showed slight anisocytosis and macrocytosis and polychromasia The erythrocyte osmotic fragility was normal Bone marrow biopsy revealed active erythroblastic

hyperplasia The serological tests for paroxysmal nocturnal hæmo globinuria were clearly positive

Further Progress The patient was next seen in 1946 She was slightly more anæmic than previously (Table 28) but otherwise her condition was unchanged Hæmoglobinuria was present more often than not but almost always this had been confined to the night time She had noticed that infections seemed to precipitate attacks during which the hæmoglobinuria might be continuous and that exposure to cold and her menstrual periods also sometimes seemed to cause exacerbations

In March 1947 she was treated with a series of transfusions of washed corpuscles at short intervals and experienced an excellent remission (Fig 98) Since then her hæmoglobin concentration has been fairly well maintained by means of transfusions with saline washed cells usually at about two monthly intervals The transfusions have almost always been well tolerated She has in this way been enabled to lead a moderately active life On one occasion however the undetected presence of anti *Kell* led to a severe hæmolytic reaction from which nevertheless she made a good recovery

She has been troubled on several occasions by phlebitis of the super ficial veins of her legs Her renal function seems to remain unimpaired despite the frequency of hæmoglobinuria She tends to develop bronchitis in the winter associated with bronchospasm

Summary A typical case of severe paroxysmal nocturnal hæmo globinuria of 14 years duration Since 1947 the patient has been kept in fair health by means of transfusions of saline washed erythrocytes

Case Report Paroxysmal Nocturnal Hæmoglobinuria

Case 34 The patient (W P) is a man now aged 58 years Hæmo globinuria dates from 193– when he had an attack lasting seven days From then until 1941 he had occasional attacks lasting a week or more usually at intervals of several months Many of them followed infec tions However he was not seriously inconvenienced and remained at work In 1941 he was admitted into hospital with hæmoglobinuria and jaundice and the diagnosis of paroxysmal nocturnal hæmoglobinuria was then established

Physical Examination He was a heavily built tall man somewhat pale and just visibly jaundiced There were no abnormal physical signs Urine passed at night contained hæmoglobin but the day time urine appeared normal

Laboratory Findings (1941) His erythrocyte count varied between 3 400 000 and 4 400 000 cells per c mm with 3 to 14% reticulocytes The leucocyte count varied between 2 500 and 2 800 cells per c mm The erythrocyte osmotic fragility was normal The acid serum test was positive a maximum of 40% of the erythrocytes being hæmolysed at the optimum pH

Further Progress He experienced more hæmoglobinuria in the winter of 1942 and had an episode lasting four days in April 1943 He was not seen again until December 1947 when he stated that he had not had any hæmoglobinuria since the attack in April 1943

In December 1947 he was found to be moderately anæmic with 4 500 000 erythrocytes per c mm 10 3 g hæmoglobin per 100 ml 1 9% reticulocytes and $<$0 5 mg bilirubin per 100 ml The acid serum

test was positive but weakly so only 13% of the corpuscles being hæmolysed at the optimum *p*H By April 1948 he was less anæmic (Hb 13 2 g per 100 ml) but the acid serum test was still weakly but definitely positive He had not had any more hæmoglobinuria

He was seen again in July 1949 three weeks after an attack of pneumonia This had not provoked any hæmoglobinuria the hæmoglobin concentration was 1[illegible] 7 g per 100 ml with 2 4% reticulocytes The acid serum test was very weakly positive

He was not seen again until September 195[illegible] Except for angina pectoris he had kept well in the meanwhile and had had no hæmoglobinuria The hæmoglobin concentration had risen to 16 5 g per 100 ml with 4 600 000 erythrocytes per c mm P C V 47% and [illegible]3% reticulocytes There were 3 600 leucocytes per c mm and 130 000 platelets per c mm The serum bilirubin concentration was 0 3 mg per 100 ml (Table 28) The acid serum test was negative and his clotted blood had not undergone lysis even after 18 hours incubation at 37 C

Summary A case of paroxysmal nocturnal hæmoglobinuria of moderate severity Hæmoglobinuria ceased after 13 years 20 years after the onset clinical and hæmatological recovery appeared complete

Case Report Paroxysmal Nocturnal Hæmoglobinuria (sine Hæmoglobinuria) Associated with Congenital Aplastic Anæmia (Fanconi)

Case 3 The patient (A H) is now aged 33 years In 1933 when aged twelve years he was admitted to King s College Hospital where a diagnosis of aplastic anæmia was made His younger brother was also affected the history of the two boys up to 1942 being described by Dacie and Gilpin (1944)

In 1939 the blood of A H was observed to undergo rapid spontaneous autohæmolysis and this observation led to the discovery that his erythrocytes behaved *in vitro* in exactly the same way as did P N H corpuscles The patient however never had any attacks of hæmoglobinuria In February 1939 splenectomy was performed—laparotomy had been primarily undertaken in an attempt to explain recurrent attacks of severe abdominal pain

Further Progress By October 1942 the P N H abnormality was less marked as judged by *in vitro* tests only about 5% of the erythrocytes being hæmolysed in acidified serum compared with [illegible]% in 1939

The patient was next seen in October 1946 the acid serum test was then only doubtfully positive In 1949 and 1951 the test was negative and (in 1951) the patient s corpuscles were shown not to be unduly sensitive to hæmolysis by anti A

Summary A case of paroxysmal nocturnal hæmoglobinuria (*sine* hæmoglobinuria) associated with (?) congenital aplastic anæmia Slow but permanent improvement leading to complete recovery followed splenectomy Laboratory tests for the P N H abnormality were positive in 194 three years after the diagnosis of paroxysmal nocturnal hæmoglobinuria doubtful in 1946 and negative in 1949 and 1951

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CHAPTER 17

HÆMOLYTIC DISEASE OF THE NEWBORN

Synonyms Erythroblastosis fœtalis icterus gravis neonatorum hæmolytic anæmia of the newborn hydrops fœtalis

History The early history of hæmolytic disease of the newborn has been well reviewed by Diamond Blackfan and Baty (1932) Hawksley and Lightwood (1934) and Pickles (1949) Although hydrops fœtalis had been recognized for centuries icterus gravis was not separated from other causes of neonatal jaundice until comparatively recent times Now it is realized that hydrops fœtalis hæmolytic anæmia of the newborn and icterus gravis are all variants of the same disease (Hawksley and Lightwood 1934 Gilmour 1944) The syndrome is now most commonly referred to as *hæmolytic disease of the newborn* The term erythroblastosis fœtalis which until recently was widely used to describe the whole syndrome refers to the finding of extramedullary hæmopoiesis and normoblastæmia

Although it had been suggested previously that the hæmolysis might be brought about by an antibody antigen reaction (Darrow 1938) evidence for this was lacking until Levine and Stetson published their classic observations in 1939 They demonstrated that the serum of a patient who had given birth to a dead fœtus contained an unusual agglutinin and suggested that the fœtus had been responsible for the immunization of the mother and that the immunizing property had been inherited from the father Later Levine Burnham Katzin and Vogel (1941) showed that in the great majority of cases the factor present in the fœtus s erythrocytes which was the cause of the immunization was identical with the Rh factor which had been recently described by Landsteiner and Wiener (1940) From that point on rapid progress was made in the understanding and treatment of the disease Diagnosis was facilitated by the use of the antiglobulin test (Coombs Mourant and Race 1945) and treatment improved by the introduction of exchange transfusion using Rh negative blood (Wallerstein 1946) It was soon realized too that hæmolytic disease of the newborn also occurred spontaneously in animals

or could be brought about by experimental immunization Study of the hæmolytic syndromes in animals has helped in the under standing of the human variety of the disease (Coombs 1950 Young *et al* 1951)

CLINICAL FEATURES

Hæmolytic disease of the newborn is a disorder which is very variable in its severity In its most severe form it causes the death of the fœtus *in utero* usually at about the 34th week of pregnancy When this happens the infant will be found on delivery to be macerated and often grossly œdematous (hydrops fœtalis) Intra uterine death seldom takes place before the 28th week (Glass 1949 Mollison and Cutbush 1954)

At the other end of the scale of severity the infant may appear clinically to be perfectly healthy at birth and during the neonatal period and to show no signs of anæmia or excessive jaundice Between these extremes every grade of severity may be encountered

Severely affected infants may be born jaundiced and seriously anæmic with cord hæmoglobin concentrations between 5 and 10 g per 100 ml They are often œdematous and have raised venous pressures and many die apparently of heart failure (Mollison and Cutbush 1949)

Less severely affected infants may appear normal or almost normal at birth Slight jaundice may be present but this is not constant usually if not present at birth it appears within a few hours Jaundice is generally well established at 24 hours (in contrast to normal infants which are at the most only very slightly jaundiced at this time) The jaundice then increases rapidly in intensity and the serum bilirubin concentration may reach peak values of 20 mg per 100 ml or more within three days of birth if the infant is untreated Thereafter if the infant survives the jaundice gradually diminishes

Although the cord hæmoglobin concentration may be within the normal range at birth (Mollison and Cutbush 1951 1954) the hæmoglobin of most affected infants falls rapidly after the first day or so of life Clinical anæmia is usually obvious by the second day

The infant s spleen and liver are palpable in most cases Cutaneous purpura and/or bleeding from the mucous membranes have been observed on rare occasions

Kernikterus There is a risk of kernikterus developing in infants that become deeply jaundiced Signs of neurological

damage appear usually 36 hours or more after birth. An affected infant becomes drowsy and may develop opisthotonos and muscular twitching (Claireaux 1950 Gerrard 1952) Death usually follows from respiratory failure This clinical syndrome is associated with bile staining of the basal cerebral nuclei (see *Pathology* p 458) The earlier the symptoms appear the worse is the prognosis (Gerrard 1952 Armitage and Mollison 1953)

The development of kernikterus has been shown to be directly related to the intensity of sensitization and the degree of immaturity of the infant (Vaughan Allen and Diamond 1950) and with the severity of the infant s anæmia (Armitage and Mollison 1953) It is also directly correlated with the depth of jaundice (Hsia Allen Gellis and Diamond 1952)

Familial Incidence Before its pathogenesis was understood hæmolytic disease of the newborn was recognized often to affect several children of the same family it was also realized that the firstborn usually escaped (Hawksley and Lightwood 1934) The familial incidence is now easily explained for once immunization has occurred a fresh pregnancy inevitably excites renewed antibody development if the father is homozygous for the immunizing antigen (see *Pathogenesis* p 458) The result is that in some families there is a tendency for successive infants to be more and more severely affected

HÆMATOLOGICAL FINDINGS

At Birth (Cord Blood)

Hæmoglobin The hæmoglobin concentration in the cord blood of many infants affected with hæmolytic disease of the newborn is often abnormally low nevertheless of Mollison and Cutbush s (1951) series of 95 infants almost exactly half had hæmoglobins exceeding 13 6 g per 100 ml the lower limit of the hæmoglobin range of the authors control series of normal infants Schulman and Smith (1954) have recently shown that the proportion of fœtal to adult hæmoglobin at birth is usually lower than normal in hæmolytic disease of the newborn In affected infants with normal hæmoglobin concentrations the absolute amount of adult hæmoglobin was increased Schulman and Smith associated this with effective regeneration

Erythrocytes The erythrocyte count of cord blood parallels the hæmoglobin content Some infants are not anæmic and have erythrocyte counts as high as 5 500 000 cells per c mm On the other hand the total count may be less than 2 000 000 cells per

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tration is much higher in infants affected with hæmolytic disease of the newborn than in normal infants there is a considerable overlap and by itself an estimation of bilirubin may fail to help in diagnosis

Changes in the Blood Picture after Birth

Progress of Anæmia The onset and rapid progress of anæmia after birth has already been referred to However the rate at which the hæmoglobin concentration and erythrocyte count fall is variable Often the fall is not great for the first two days but from the third day to the end of the first week the rate of fall may even be as great as 3 g hæmoglobin per 100 ml in 24 hours

As the supply of antibody from the mother is cut off from the time of birth onwards the rapidly increasing anæmia after birth needs some explanation It seems unlikely that a significant amount of antibody is absorbed from colostrum or milk (see p 461) The most likely explanation for the progressive anæmia is a diminution in the rate of compensatory erythropoiesis which up till birth had been more or less keeping pace with the hæmolysis The high normoblast and reticulocyte counts fall swiftly after the first day or two to quite low levels and as blood destruction appears to proceed unabated for some time in untreated infants anæmia rapidly develops The failure in erythropoiesis is probably the result of the increase in oxygen tension in the blood after birth compared with intra uterine levels

The Increase in Serum Bilirubin As mentioned on p 452 the serum bilirubin concentration of an infant affected with hæmolytic disease of the newborn increases more rapidly and reaches far higher levels than in normal healthy infants The marked increase in the bilirubin concentration of affected infants is due to two factors continuing hæmolysis and the inability of the liver of the newborn infant to excrete bilirubin rapidly In untreated affected infants the peak bilirubin concentration is usually reached during the third day of life the average concentration at this time in the series of Hsia and co workers (1952) being 30 mg per 100 ml

SEROLOGY

Direct Antiglobulin Test Cord blood erythrocytes always give a positive antiglobulin test in hæmolytic disease of the newborn due to Rh antibodies The intensity of the reaction varies

c mm in infants born dead or moribund Typically there is marked macrocytosis and the M C V and M C D are higher than in normal newborn infants of comparable maturity Stained films show anisocytosis with many macrocytes and a small degree of poikilocytosis Most of the largest macrocytes are polychromatic Reisner (1943) constructed Price Jones curves and described the presence of two peaks a normocytic peak and a macrocytic peak or two macrocytic peaks Spherocytosis is not usually obvious in hæmolytic disease of the newborn due to immunization against Rh antigens when due to anti A on the other hand this may be a marked feature of the film (see p 463) As a rule moderate numbers of siderocytes are present (mean 8 7% range 0 to 35% Douglas and Dacie 1953)

Normoblastæmia Excessive numbers of normoblasts are almost invariably found in cord blood films of infants with hæmolytic disease of the newborn They are mostly mature or almost mature normoblasts and macronormoblasts but in the more severely affected infants early polychromatic or even basophilic normoblasts are often present in large numbers True megaloblasts are never present The total number of normoblasts may easily exceed the leucocyte count and in some cases as many as 100 000 per c mm may be present Even in the mildest cases the number of normo blasts usually exceeds that normally found in full term newborn infants i e up to 10 per 100 leucocytes (Mollison 1951)

Reticulocytes The reticulocyte count is characteristically raised In severely affected infants the count may be as high as 50% and even in the mildest cases it is generally higher than normal i e >5%

Leucocytes The leucocyte count may be as high as 30 000 per c mm Neutrophils predominate and small numbers of immature cells may be present (Blackfan Diamond and Leister 1944)

Platelets The platelet count is usually low in severe cases (Blackfan Diamond and Leister 1944)

Osmotic Fragility Osmotic fragility is usually normal in hæmolytic disease of the newborn due to anti Rh However it may be increased in severely affected infants (Crawford Cutbush and Mollison 1953) In hæmolytic disease of the newborn due to anti A on the other hand increased fragility seems to be found regularly (Robinson Phillips and Prystowsky 1951 Crawford Cutbush and Mollison 1953)

Serum Bilirubin The bilirubin concentration in cord serum varies from 1 mg to more than 9 mg per 100 ml (Mollison and Cutbush 1949 Hsia *et al* 1952) Although the average concen

PATHOLOGY

The macroscopic and microscopic findings in infants who have died of hæmolytic disease of the newborn have been the subject of many detailed reports (e g Hawksley and Lightwood 1934 Gilmour 1944 Pickles 1949 Lindsay 1950) The main features are as follows The infant is usually pale and jaundiced and may be œdematous—the œdema is severe and generalized in hydrops fœtalis serous effusions are often present Petechiæ are sometimes found on the serous surfaces and larger hæmor rhages are not uncommon in the lungs The liver and the spleen are invariably enlarged sometimes markedly so An increase of suprarenal cortical lipoid was noted by Gilmour (1944) in hydrops cases The brain may be stained with bile pigment in deeply jaundiced infants the basal nuclei being particularly affected (kernikterus Schmorl 1904) The placenta is often bulky and pale

Histology The most striking histological feature is the widespread extramedullary erythropoiesis the infant s reaction to severe hæmolysis This was referred to by Rautmann (1912) as erythroblastosis fœtalis The extramedullary erythro poiesis is more widespread than would be supposed on naked eye examination In addition to being present in the liver and spleen it has been observed for instance in the pancreas kidneys adrenals lymph nodes gonads and placenta and even in connec tive tissue and skin (Hawksley and Lightwood 1934 Gilmour 1944)

Liver Sections show a variable but often enormous proliferation of normoblasts within the liver sinusoids compressing and displacing the liver parenchyma cells Fatty infiltration is not uncommon and focal necroses may be seen A considerable amount of bile pigment is found either as fine droplets in liver cells or as thrombi in the canaliculi particularly in jaundiced infants dying after birth An increase in reticulin has been reported (Gilmour 1944) but fibrosis is incon spicuous as a rule The degree of siderosis varies

The liver structure (and function) eventually return to normal in infants who survive Hawksley and Lightwood (1934) noted that the liver of an infant dying (of pertussis) ten weeks after recovery from hæmolytic disease of the newborn was normal except for the presence of a few normoblasts and some hæmosiderin

Spleen Sections show congestion and widespread hæmopoiesis The Malpighian bodies are small Bile pigment and hæmosiderin may be found in infants who die in the neonatal period Excess hæmosiderin is however not always found

Bone marrow All the medullary cavities present at birth contain

Usually the reaction is strong well marked agglutination taking place within 30 seconds of the addition of the antiglobulin serum Weak reactions (due to anti Rh) are usually associated with clinically mild forms of the disease (Pickles 1949 Mollison 1951) Maximum agglutination characteristically takes place in comparatively highly diluted antiglobulin serum (1 in 64 to 1 in 256) if a highly potent serum is used In contrast the direct antiglobulin reaction is usually weak in hæmolytic disease of the newborn due to anti A it may even be negative (Crawford Cutbush and Mollison 1953) (see p 463)

The direct antiglobulin reaction often remains positive for many weeks after birth in infants suffering from hæmolytic disease due to Rh antibodies although the intensity of the reaction weakens gradually *Mollison and Cutbush (1949) obtained positive* reactions in one case up to 93 days after birth However the reaction usually becomes negative within a few days in infants effectively treated by means of exchange transfusion with Rh negative blood

The persistence for many weeks of sensitized erythrocytes in the circulation of an untreated infant shows that a moderate degree of coating with antibody is not incompatible with a normal or almost normal cell survival

Antibody in the Infant s Serum Rh antibody can usually be detected in an affected infant s serum at the time of birth Using the indirect antiglobulin test or the albumin technique or both, Mollison and Cutbush (1949) were able to detect the presence *of antibody in 35 out of 41 cases However the amount (titre)* of *free* antibody did not seem to be correlated with the severity of the hæmolysis as judged clinically (Mollison and Cutbush 1949 Sturgeon 1954)

The antibody may persist for a surprisingly long time It may be detected in a gradually declining concentration for weeks in infants given repeated transfusions of Rh negative blood it has been detected for four months or more in the sera of Rh negative infants born to previously sensitized mothers whose sera contain anti Rh (Wiener *1948 Mollison 1951) A straight line is* obtained if the antibody titre is plotted on a logarithmic scale against time (Mollison 1951)

Free antibody may also be detected at birth or shortly afterwards in the serum of infants with hæmolytic disease of the newborn due to anti A Crawford Cutbush and Mollison (1953) using the antiglobulin method obtained positive results in eight out of eleven cases

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effective stimulus to an antibody forming mechanism which had already been primed by the first pregnancy However affected infants are far less common than might be expected on theoretical grounds and many Rh negative women married to homozygous Rh positive husbands have several children without any being obviously affected with hæmolytic disease Mollison (1952) has made some interesting calculations ten out of every 100 children are likely to be Rh positive (and to have an Rh negative mother)—these are the children at risk Yet the incidence of hæmolytic disease of the newborn is about one affected child in 170 to 200 pregnancies (Schwartz and Levine 1943 Boorman Daley and Dodd 1947) only one seventeenth to one twentieth of the theoretical maximum Many of these births are of course first pregnancies in which the risk is very slight indeed Even so the incidence is far less than might be expected

It cannot be claimed that the exact mechanism of the immunization is completely understood nor is it clear why immunization so often fails to occur It is known that Rh negative women (and men) vary in their power of responding to the stimulus of Rh positive blood given intravenously Waller (1949) reported that three out of ten volunteers developed antibodies after three 2 ml injections had been given at intervals of six weeks after six injections nine out of the ten had responded Wiener (1949) injected larger numbers 54% of 47 volunteers responded after three injections at intervals of three to four months but only two thirds of them had responded at the end of five injections This variability in response must clearly be one factor which affects the development of antibodies when there is incompatibility between the blood groups of the mother and her fœtus Other possible factors including the role of the placenta are considered by Coombs (1950)

It is still unknown whether the immunizing antigen is conveyed to the mother in a soluble form or by intact erythrocytes or even by fragments of placental tissue making their way into the maternal circulation (Levine 1948 Kline 1949) Mollison (1951) concluded that intact erythrocytes were unlikely to be responsible and favoured the other two hypotheses It is possible that the primary sensitization is to a large extent brought about by events associated with parturition when the chances of fœtal placental tissue or erythrocytes entering the maternal circulation are likely to be at their greatest There is evidence however that a first pregnancy terminating by abortion at ten to twelve weeks may act as a sufficient primary stimulus for serious immunization to develop

active hæmopoietic tissue Erythropoiesis predominates normoblasts in all stages of development being present in vast numbers

Brain Degeneration and disappearance of ganglion cells early gliosis and the appearance of fat granule cells have been described in the cerebral nuclei of infants dying of kernikterus (Gilmour 1944 Claireaux 1950)

ÆTIOLOGY AND PATHOGENESIS

Hæmolytic disease of the newborn is now known to be the result of incompatibility between the blood group antigens of the fœtus and those of the mother This is possible because the fœtus receives half its complement of blood group determining genes from the father Differences between fœtus and mother *are in fact almost inevitable because of the existence of so many* blood group antigens

Fortunately however only a proportion of the blood group antigens which the fœtus may possess commonly stimulate the mother to form iso antibodies should she lack the corresponding antigens in her own erythrocytes D is by far the most important of the Rh antigens which cause hæmolytic disease of the newborn but other antigens such as E c C and C^w have been implicated on rare occasions (Lawler and van Loghem 1947 van Loghem and Hart 1949 Pickles 1949 Malone and Dunsford 1951 Race 1952 Grundorfer 1953 van Loghem and Bakx 1953) *Kell and S incompatibility have also been known to cause hæmo*lytic disease (Mollison 1951 Levine Ferraro and Koch, 1952) Cases due to ABO incompatibility almost all due to anti A also occur (see p 462) numerically they are the second most important type Rarely *hæmolytic disease has followed immunization* to the exceedingly uncommon private blood factors (Levine *et al* 1951)

Even when an Rh negative woman lacking the D antigen marries a man homozygous for D and bears in consequence a fœtus containing the D antigen, immunization and the development of anti D is the exception rather than the rule It is exceedingly uncommon for the first child of such a marriage to be affected when this happens it usually transpires that the woman has received one or more transfusions (Diamond 1945 Levine and Waller 1946) *or has been given intramuscular injections of* blood perhaps many years previously (Bessis 1947 Levine Vogel and Rosenfield 1953)

The usual course of events is for a healthy child to be born at the first pregnancy and then for the second or the third child to be affected the later pregnancies presumably providing an

effective stimulus to an antibody forming mechanism which had already been primed by the first pregnancy However affected infants are far less common than might be expected on theoretical grounds and many Rh negative women married to homozygous Rh positive husbands have several children without any being obviously affected with hæmolytic disease Mollison (1952) has made some interesting calculations ten out of every 100 children are likely to be Rh positive (and to have an Rh negative mother)—these are the children at risk Yet the incidence of hæmolytic disease of the newborn is about one affected child in 170 to 200 pregnancies (Schwartz and Levine 1943 Boorman Daley and Dodd 1947) only one seventeenth to one twentieth of the theoretical maximum Many of these births are of course first pregnancies in which the risk is very slight indeed Even so the incidence is far less than might be expected

It cannot be claimed that the exact mechanism of the immunization is completely understood nor is it clear why immunization so often fails to occur It is known that Rh negative women (and men) vary in their power of responding to the stimulus of Rh positive blood given intravenously Waller (1949) reported that three out of ten volunteers developed antibodies after three 2 ml injections had been given at intervals of six weeks after six injections nine out of the ten had responded Wiener (1949) injected larger numbers 54% of 47 volunteers responded after three injections at intervals of three to four months but only two thirds of them had responded at the end of five injections This variability in response must clearly be one factor which affects the development of antibodies when there is incompatibility between the blood groups of the mother and her fœtus Other possible factors including the role of the placenta, are considered by Coombs (1950)

It is still unknown whether the immunizing antigen is conveyed to the mother in a soluble form or by intact erythrocytes or even by fragments of placental tissue making their way into the maternal circulation (Levine 1948 Kline 1949) Mollison (1951) concluded that intact erythrocytes were unlikely to be responsible and favoured the other two hypotheses It is possible that the primary sensitization is to a large extent brought about by events associated with parturition when the chances of fœtal placental tissue or erythrocytes entering the maternal circulation are likely to be at their greatest There is evidence however that a first pregnancy terminating by abortion at ten to twelve weeks may act as a sufficient primary stimulus for serious immunization to develop

at a second pregnancy Nevertheless here too it is possible that it is fœtal tissue escaping into the maternal circulation at the time of the miscarriage that acts as antigen

The Maternal Antibodies

Most commonly a sensitized Rh negative woman develops incomplete anti D occasionally ' complete in saline agglutinating anti D or anti C or anti E may be present in addition Anti E rarely and anti C or anti C^w very rarely occur by themselves in the sera of pregnant women (Malone and Dunsford 1951 van Loghem and Bakx 1953) Antibodies are not usually detectable until about the fifth month of pregnancy when developed for the first time they usually increase in titre as pregnancy proceeds and they are almost invariably detectable by the 34th week if the fœtus is affected When antibodies have been present in a previous pregnancy they may be detected in a succeeding pregnancy from the earliest stages sometimes they increase in titre but more often than not the titre remains unchanged (Wiener Nappi and Gordon 1951b Arnold Walsh and Herzger 1951) When in the succeeding pregnancy the fœtus is Rh negative (husband heterozygous for D) the antibodies nevertheless persist throughout pregnancy (Davidsohn and Stern 1948 Wiener Nappi and Gordon 1951a) they may even increase slightly in titre (Pickles 1949 Schneider *et al* 1950)

The titre of the antibodies varies greatly from case to case Mollison and Cutbush (1949) reported titres ranging between 1 and 16 000 in 48 patients There is probably some general but not very close correlation between the concentration of antibodies and the clinical severity of the resultant disease in the affected infant (Mollison and Cutbush 1949 Allen Diamond and Vaughan 1950a Wiener Nappi and Gordon 1952) After delivery the highest titres are found in the first week or so of the puerperium Thereafter the concentration of antibodies gradually falls often however they can be detected many years subsequently (Wiener Nappi and Gordon 1951b) It is also known that when in saline agglutinating antibodies predominate the outlook is more favourable than when incomplete (blocking) antibodies are present alone at comparable titres (Davidsohn and Stern 1948 Mollison 1951) As will be referred to in the next paragraph the in saline agglutinating type of antibody probably fails to cross the placenta

Passage of Antibodies through the Placenta It has been known for a considerable time that the placenta is permeable to

many types of antibody (McKhann and Kapnick 1938) It has also been shown that antibodies and gamma globulins are present in fœtal serum in much higher concentrations towards the end of pregnancy than in the first and second trimesters (Vahlquist Lagercrantz and Nordbring 1950 Moore Du Pan and Buxton 1949)

However only the incomplete form of anti Rh seems to have the power of crossing the placenta

When the maternal serum contains antibodies and the fœtal erythrocytes are Rh negative (father heterozygous for D) the titre of incomplete antibodies in the cord serum closely parallels the titre in the maternal serum (Wiener 1948 Mollison 1951) On the other hand when complete and incomplete forms exist together in the maternal serum only the incomplete form will be found in the fœtal serum (Broman 1948) Incomplete antibody is capable of crossing the placenta at a surprisingly early stage of pregnancy Mollison (1951) reported finding that the direct antiglobulin test was positive in two fœtuses of apparently ten and sixteen weeks of age respectively

In saline agglutinating anti A (and anti B) cross the placenta barrier with difficulty and may not be demonstrable in cord serum (Tovey 1945 Wiener Wexler and Hurst 1949) The incomplete and probably hæmolytic forms of the antibody certainly cross far more easily (see p 463)

Transference of Antibody in Colostrum and Milk

There is some evidence for the presence of Rh antibodies in human milk and colostrum (Witebsky and Heide 1943) but no conclusive evidence that they can be absorbed by the infant even in the first days of life (Cathie 1947)

In dogs on the other hand there is no evidence that transplacental transfer of antibody from mother to puppy takes place The colostrum however contains antibodies and in experimentally immunized bitches severe hæmolytic disease of the newborn develops if the puppies are allowed to suckle their mothers during the first day of life (Young *et al* 1951)

Mechanism of Erythrocyte Destruction

As already discussed on p 299 the mechanism by which incomplete antibodies cause erythrocyte destruction *in vivo* is not completely understood *In vitro* antibodies such as anti D cause at the most very small amounts of hæmolysis and they do not seem to be potent in promoting erythrophagocytosis In hæmolytic disease of the newborn the almost invariable absence of marked hæmoglobinæmia indicates that hæmolysis occurs for the most part outside the circulating blood stream but exactly how and in what organs is obscure

Possibly autohæmagglutination resulting from sensitization is important in areas where the circulation is slow and conceivably the

metabolism of the erythrocyte membrane is affected deleteriously by the adsorption of antibody globulin (see also p 302) It is also possible that phagocytosis of sensitized corpuscles is more important than appears from tests *in vitro* Erythrophagocytosis has been detected for instance in the peripheral blood films of affected infants (Cooper 1950) In severe cases too Schumm s test for hæmatin may be positive and osmotic fragility increased Increased fragility probably indicates a severe degree of damage by antibody and in infants in which this is found it is likely that a certain amount of lysis takes place in the blood stream

In hæmolytic disease of the newborn due to anti A comparable although not identical mechanisms are probably at work

HÆMOLYTIC DISEASE OF THE NEWBORN DUE TO ANTI A (OR ANTI B)

It is now known with certainty that incompatibility within the ABO groups is an important cause of hæmolytic disease of the newborn Although this had been suspected by Levine and his colleagues in 1941 conclusive studies have been carried out only in recent years (Grumbach and Gasser 1948 Boorman Dodd and Trinick 1949 Wiener Wexler and Hurst 1949 Crawford Cutbush and Mollison 1953)

Hæmolytic disease of the newborn due to anti A is a less common disorder than that due to anti Rh and it differs from it clinically as well as hæmatologically and serologically

Clinical Features

These are well summarized by Wiener Wexler and Hurst (1949) and by Mollison (1951) One difference from hæmolytic disease of the newborn due to anti Rh is that infants born of first pregnancies are not infrequently affected According to Mollison (1951) first born infants were affected in thirteen out of 33 families in which the disorder had occurred Mollison attributed this to the fact that many heterogenetic stimuli such as injections of tetanus toxoid and T A B vaccine stimulate the formation of the immune type of anti A in group O subjects

The infants as a rule are not seriously affected Anæmia at birth is absent or minimal and there is seldom a substantial fall in hæmoglobin subsequently Jaundice however develops rapidly after birth and kernikterus has been described (Grumbach and Gasser 1948 Levine Vogel and Rosenfield 1953)

Blood Picture and Serology

The hæmatological and serological findings in eleven carefully studied cases were recently described in detail by Crawford

Cutbush and Mollison (1953) Anæmia was absent or mild but the M C H C tended to be higher than in normal infants and infants with hæmolytic disease due to anti Rh The reticulocyte count varied from 8 to 21% on the first day of life excessive normoblasts (30 normoblasts or more per 100 leucocytes) were found in seven out of nine infants jaundice was moderate or marked (maximum values 7 mg to 26 mg per 100 ml) Spherocytes could be seen in the infants blood films and definite increases in osmotic fragility were demonstrated in ten of the eleven cases The finding of increased fragility is in strong contrast to hæmolytic disease of the newborn due to anti Rh in which an increase in fragility is seldom found

The direct antiglobulin test was positive in seven out of the eleven cases the reactions were far weaker than those usually found in hæmolytic disease due to anti Rh Free anti A was detected in the cord serum of eight infants the antibody however only sensitized A_1 cells to antiglobulin serum and did not cause agglutination or hæmolysis All the mothers were group O their sera contained immune anti A which sensitized A_1 cells to antiglobulin serum as well as hæmolysing them in the presence of complement The agglutinin titres were mostly high but not exceptionally so

Crawford Cutbush and Mollison (1953) concluded that it was the immune type of anti A in the maternal sera (i e antibody with marked hæmolytic and sensitizing properties) that was responsible for the hæmolysis in the infant They suggested that if a mother s serum failed to hæmolyse her infant s erythrocytes *in vitro* this could be taken as evidence against the possibility that hæmolytic disease in the infant could have been due to anti A

Recently it has been pointed out by Rosenfield (1954) that in hæmolytic disease due to ABO incompatibility the mother has been almost invariably group O not group B This has been explained on the hypothesis that the active antibody is the cross reacting anti C which only group O subjects can form and which possibly passes the placental barrier more easily than do anti A or anti B (see Wiener Samwick Morrison and Cohen 1953)

DIAGNOSIS OF HÆMOLYTIC DISEASE OF THE NEWBORN

Hæmolytic disease of the newborn is strongly suggested if an infant develops rapidly deepening jaundice within a few hours of

birth associated with anæmia marked reticulocytosis and normoblastæmia particularly if there is a history of similar occurrences in previous pregnancies or of unexplained stillbirths Certain confirmation of the diagnosis can only be made by serological means

The most significant single serological observation in hæmolytic disease of the newborn due to all types of incompatibility is the demonstration of sensitization of the infant s corpuscles by means of the antiglobulin test It is only in hæmolytic disease due to anti A that the test is at all likely to be negative The demonstration of antibodies in the maternal serum capable of agglutinating or sensitizing her infant s corpuscles supports the diagnosis of hæmolytic disease of the newborn The presence of antibodies in the maternal serum is by itself not diagnostic for they may have been formed as the result of a previous pregnancy or transfusion and not as the result of incompatibility in the pregnancy in question

If there appears to be no obvious group incompatibility between mother and child e g if both are Rh negative or Rh positive then in the presence of obvious signs of neonatal hæmolysis *sensitization against antigens other than D such as c S or Kell* and ABO incompatibility must be thought of

Other causes of neonatal jaundice and of anæmia with or without erythroblastosis exist Congenital liver disease (obliteration of the bile ducts cirrhosis or hepatitis) congenital syphilis and congenital hæmolytic anæmias such as hereditary spherocytosis are possibilities which should be borne in mind In no instance however is the clinical evolution of the case just the same as in typical hæmolytic disease of the newborn in no instance too will the infant s corpuscles be found to be sensitized and to be agglutinated by antiglobulin serum

TREATMENT

Two main aspects have to be considered (1) the management and treatment of the established disease in the infant and (2) the attempts that have been made to protect the fœtus *in utero* by reducing antibody formation in the mother

Treatment of the Infant by Blood Transfusion

The aims of transfusion therapy are to correct any existing anæmia to safeguard the infant against the severe anæmia which may develop during the first week or two of life and to prevent the development of severe degrees of jaundice which might lead

to kernikterus When the antibody is anti D these aims are best accomplished by exchange transfusion with Rh negative blood carried out during the first day of life Simple transfusion with Rh negative blood repeated if necessary can effectually compensate for anæmia it does not however prevent deep jaundice developing Exchange transfusion prevents jaundice by taking from the infant a large proportion of its corpuscles which are already sensitized and destined to be rapidly destroyed Exchange transfusion is nevertheless a rather elaborate procedure and needs to be carefully carried out and in very mild cases it is probably unnecessary In any case it can be carried out easily only through the umbilical vein and this usually restricts its use to the first 24 hours of life The selection of cases for exchange transfusion and further details of the method are considered below

Exchange Transfusion Wallerstein (1946) seems to have been the first to realize that withdrawal of part of the infant s blood at the same time as a transfusion of Rh negative blood might be of real value in the treatment of hæmolytic disease of the newborn Wiener and Wexler (1946) modified Wallerstein s technique by bleeding the infant from the radial artery instead of from the sagittal sinus Diamond (1947) and Diamond Allen and Thomas (1951) improved the method further by showing that exchange transfusion could be quite simply carried out if a plastic catheter was inserted into the umbilical vein Details of the umbilical vein method were given by Mollison and Cutbush (1948) and by Mollison (1951 1952) Only a brief description of the technique will be given here

The recommended method is to use a 20 ml syringe attached to a three way tap and starting by the withdrawal of 20 ml of the infant s blood alternately to withdraw blood from the infant and replace it by a concentrated suspension of normal Rh negative erythrocytes in citrated plasma (Hb content at least 15 g per 100 ml) until about 60 ml of blood per lb weight of the infant have been withdrawn and replaced In most cases the infant will then be left with a venous hæmatocrit of 50% 90% of the blood being the donor s (Rh negative) corpuscles and only 10% that of the infant This degree of exchange ensures that the infant will not become so anæmic in subsequent weeks as to require further transfusion A nomogram for calculating the exact volume of blood to be exchanged knowing the infant s weight the hæmatocrit of the infant s venous blood and that of the blood to be transfused was published by Veall and Mollison (1950)

Exchange Transfusion *v* Simple Transfusion The superiority of the exchange transfusion over simple transfusion is now firmly established (Allen Diamond and Vaughan 1950b

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vein and amounts up to 100 ml may be transfused After the first 24 hours it will be necessary either to insert a cannula into the internal saphenous vein at the ankle and allow up to 150 ml or even 200 ml of blood to run in at a slow drip rate or to attempt a scalp vein transfusion using a syringe A good account of these methods the amounts that may be given and suggested rates of administration are given by Mollison (1952)

Indications for Transfusion

The problem of when and how to transfuse has already been discussed To recapitulate exchange transfusion is the method of choice all premature affected infants should be treated irrespective of their cord hæmoglobin concentrations as well as all mature affected infants with cord hæmoglobins less than 15 5 g per 100 ml mature infants with cord hæmoglobins between 15 5 g to 17 5 g per 100 ml should be treated if they become jaundiced within the first 24 hours (particularly if male) mature infants whose cord hæmoglobins exceed 17 5 g per 100 ml need not be treated Mollison and Cutbush (1954) made the additional point that immediate exchange transfusion should be undertaken if the mother has previously given birth to an affected child irrespective of any other indication Jones Diamond and Allen (1954) stressed the desirability of carrying out a second or even a third exchange transfusion within the first 48 to 72 hours if the serum bilirubin still rises a concentration of 20 mg per ml being deemed the maximum permissible

Indications for simple transfusion after the first day of life are less clear cut The hæmoglobin concentration falls steadily even in normal infants for the first two to three months However a hæmoglobin more than 3 g below the normal concentration for the age of the infant e g 10 g or less per 100 ml at the end of the second week might be held to be an indication for transfusion In coming to a decision to transfuse it is wise to take into account the infant s general condition and whether the hæmoglobin concentration is still falling or starting to rise

Attempts to Diminish the Formation of Antibodies by the Mother

It would obviously be of great value if anything could be done to diminish the formation of antibodies by a sensitized woman Various attempts have been made employing the principle of counter sensitization i e the intensive stimulation by powerful

Mollison and Walker 1952 Armitage and Mollison 1953) The mortality as well as the incidence of kernikterus and permanent cerebral damage in survivors are reduced

Mollison and Walker (1952) who analysed the results of a controlled trial organized by the Medical Research Council concluded that it was mainly in two categories of infants that the superiority of exchange over simple transfusion was most marked (1) in severely affected mature infants (with cord hæmoglobin concentrations of 11 g per 100 ml or less) and (2) in moderately severely affected immature infants (with cord hæmoglobin concentrations exceeding 11 g per 100 ml) The mortality of slightly or moderately affected mature infants was low and that of severely affected immature infants high irrespective of the type of transfusion given

Mollison and Walker (1952) concluded that it was unwise to induce labour three to five weeks before term in an attempt to protect the infant against the mother s antibodies They found that the survival rate of affected infants deliberately delivered prematurely was lower than those whose delivery was spontaneous and not hastened in any way

Armitage and Mollison (1953) have recently published a further analysis of the British M R C trial including curves relating expected survival to cord hæmoglobin concentration and maturity or immaturity (their Fig 2) Their analysis showed that it was unwise to withhold exchange transfusion because the cord hæmoglobin concentration exceeded 15 g per 100 ml for without treatment the incidence of kernikterus was 7% in mature infants and 16% in immature ones They also concluded that it was probably best to treat all infants born prematurely by exchange transfusion

Mollison and Cutbush (1954) discussed the rather difficult problem as to what should be done for mature infants with cord hæmoglobin concentrations exceeding 15 5 g per 100 ml but less than 17 5 g per 100 ml They decided that it was probably wise to transfuse any child in this category who became clinically jaundiced within the first 24 hours particularly if the infant was a male

Simple Transfusion with Rh negative Blood As already indicated exchange transfusion through the umbilical cord is the method of choice in all cases requiring transfusion However if the apparatus or other facilities are not available simple transfusion is often better than no treatment at all During the first 24 hours after birth this is best done *via* the umbilical

in excess of that which might have been expected (Mollison and Cutbush 1954)

Geppert Akeroyd and Simpson (1953) treated twenty affected infants immediately after birth with A C T H an initial dose of 1.5 mg was given and then 0.5 mg six hourly The infants received no other treatment The results were not dramatic although the hæmoglobin concentrations tended to rise or to be maintained whilst the infants were receiving the drug only to fall when it was discontinued The strength of the antiglobulin reaction became less Three infants died (one with kernikterus) and it is obvious that although A C T H may be of some value it cannot in any way be looked upon as a substitute for exchange transfusion

PROGNOSIS AND SEQUELÆ

It is difficult to give an overall figure for the mortality caused by hæmolytic disease of the newborn for so much depends upon the severity of the disease and the treatment the infant receives The outlook for the first affected infant is good According to Allen Diamond and Vaughan (1950a) 30% show no clinical signs of disease and only a few are stillborn The prognosis for subsequent affected infants is much less favourable according to Mollison and Cutbush (1954) there is only about a 60% chance of survival

Mollison and Cutbush (1951) calculated by Probit analysis the chances of survival of 51 uniformly treated infants according to their cord hæmoglobin concentrations This analysis showed that 99 out of 100 infants born with cord hæmoglobins of 15 5 g per 100 ml should survive as compared with an expected survival of 79% of those with cord hæmoglobin of 10 g and 39% of those with 7 5 g hæmoglobin per 100 ml As referred to on p 466 the outlook is better in full term than in premature infants and better in slightly to moderately anæmic infants if they are treated by exchange transfusion rather than by simple transfusion Of a larger series of 477 infants born at term or within 35 days of term twenty four were stillborn of those born alive 79 died within the first week an overall mortality of about 22% (Mollison and Walker 1952) Some of the infants in this series were treated by exchange transfusion others by simple transfusion Still others with cord hæmoglobins exceeding 15 5 g per 100 ml received no treatment

Sequelæ

The most serious sequel in infants who have recovered from the immediate effects of hæmolytic disease of the newborn is damage

antigens of harmless antibodies in the hope that the formation of Rh antibodies might be simultaneously diminished There is some experimental evidence reviewed by Unger (1949) in favour of the possibility Unfortunately there is no evidence that this line of treatment succeeds in practice

Unger (1949) injected T A B and/or pertussis vaccine and concluded that although there was no evidence for or against the use of vaccines in unsensitized women the course of injections was *without* value in women already sensitized He also tried Rh hapten (Carter 1947) in eleven patients whose sera contained Rh antibodies but again failed to show that this was of any value in reducing the antibody concentration Negative results with Rh hapten have also been reported by Hamilton and Brockland (1950) and Spurling Sacks and Jahn (1950) Unger (1949) also carried out exchange transfusions in four pregnant women who were already sensitized In no instance did the antibody titres change appreciably despite the enormous volumes of Rh negative blood which were expended

Treatment with A C T H or Cortisone

The usefulness of A C T H or cortisone in the treatment of auto immune acquired hæmolytic anæmia has naturally led to the trial of the hormones in hæmolytic disease of the newborn It is not yet possible to assess their value The hormones have been given to the mother during pregnancy and to infants after delivery Some workers have reported that the antibody titres in the maternal serum were unaltered (Doerner *et al* 1951 Schmidt Huurman and Hansen 1953) others have reported that the titres were diminished (Christensen Margulis and Stewart 1952)

Christensen Margulis and Stewart (1952) treated ten pregnant women whose sera contained antibodies eight of whom had already given birth to affected infants The duration of treatment varied from three days to six months and as much as 6 500 mg of cortisone was given to one patient Eight infants were born alive two were stillborn The authors concluded that it was doubtful whether the infants had been benefited by the treatment their mothers had received

Hunter (1954) treated 67 women all of whose sera contained antibodies at a titre of 8 or higher most of them had previously given birth to an affected infant As a rule 100 mg of cortisone were given daily in four divided doses Hunter concluded that the stillbirth rate and neonatal death rate was significantly reduced Of thirteen patients who had previously had stillborn affected infants ten were delivered of live infants (one was Rh negative) two died subsequently This means that seven out of the twelve infants at risk lived a survival rate

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to the nervous system The early reports on the incidence of nervous disease were reviewed by Pickles (1949) and Evans and Polani (1950) In most instances mental backwardness is associated with signs of damage to the extrapyramidal system (Gerrard 1952) The incidence however is quite small Mollison and Walker (1952) found that thirteen out of 368 infants (3 6%) showed signs of damage to the nervous system at the age of one month Evans and Polani (1950) described 16 patients and reviewed 63 cases in the literature Over 80% had athetosis chorea or choreo athetosis many were mentally defective and some were deaf As already mentioned it is likely that exchange transfusion carried out in the first day of life will reduce substantially the incidence of nervous complications by the prevention of kernikterus

Other Changes Apart from involvement of the nervous system the only other relatively common sequel is a greenish discolouration of the deciduous teeth (Nickerson and Moulton 1943) Pickles (1949) reported an incidence of 6% The possibility that hæmolytic disease of the newborn may occasionally give rise to cirrhosis of the liver has been suggested on various occasions (see Pickles 1949) The association cannot yet be considered to have been proved conclusively

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centrifuged for 5 minutes at 2 000 r p m The amount of hæmolysis in each tube is then compared with that in the 100% lysis tube (0 1% NaCl) using a photoelectric colorimeter provided with a green (Ilford 625) filter The supernatant from the 0 85% NaCl tube is used as the blank Usually the supernatants can be poured by decantation into the colorimeter cell With a good colorimeter as little as 1% of lysis can be estimated

The author adds the blood to the hypotonic solutions by means of a glass capillary automatic pipette calibrated to deliver 0 05 ml With this instrument almost exactly equal amounts of blood can be added to each tube but skill and practice is needed Alternatively straight pipettes calibrated to contain 0 05 ml may be used but this method although accurate if a dry pipette is used for each addition is tedious A more rapid but far less accurate method is to add one drop of blood to each tube

Factors Affecting Osmotic fragility Tests

In carrying out fragility tests (by any method) three variables capable of markedly affecting the results must be controlled quite apart from the accuracy with which the saline solutions have been made up These are (1) the relative volumes of blood and saline (2) the final pH of the blood saline suspension and (3) the temperature at which the tests are carried out

A proportion of 1 part of blood to 100 parts of saline is convenient because not only can the resultant hæmolysis be read off directly in most colorimeters without further dilution but the concentration of blood is so small that the added plasma hardly affects the osmotic equivalence of the saline If on the other hand as much as 1 part of blood is added to say 20 parts of saline the added plasma substantially increases the effective tonicity of the hæmolysing solution However if weak suspensions of blood in saline are used it is then necessary to control the pH of the hypotonic solutions and for this reason phosphate buffer is added to the saline in the present method Even so small differences will be found between the fragility of strictly venous blood and maximally aerated blood It is recommended therefore for the most accurate results that the blood should be mixed until bright red as is achieved during defibrination Finally for really accurate work the estimations should always be carried out at the same temperature for most purposes though room temperature is sufficiently constant

The extent of the effect of pH and temperature on osmotic fragility is illustrated in the paper of Parpart and co workers (1947) The effect of pH is the more important here a shift of 0 1 of a pH unit is equivalent to altering the tonicity by 0 01% the fragility of the erythrocytes being increased by a fall in pH A rise in temperature decreases the fragility a rise of 5 C being equivalent to an alteration in tonicity of about 0 01%

Hæmolysis is virtually complete at the end of 30 minutes at 20 C and the hypotonic solutions may be centrifuged at the end of this time Parpart and his colleagues recommended the addition of complemen

CHAPTER 18

HÆMATOLOGICAL TECHNIQUES USEFUL IN THE INVESTIGATION OF HÆMOLYTIC ANÆMIAS

In this chapter will be described techniques which may be used in the investigation of hæmolytic anæmias Only methods which the author or his colleagues have used will be described and no attempt has been made to write a comprehensive text on laboratory methods

OSMOTIC FRAGILITY

The method to be described is based upon that of Parpart and co workers (1947) Hypotonic saline buffered to *p*H 7 4 with sodium phosphates is used and the blood is added to the hypo tonic solution in the proportion of 1 to 100 The test is carried out at room temperature and hæmolysis read photoelectrically

Reagents A stock solution of buffered sodium chloride (A R) osmotically equivalent to 10% NaCl is made as follows NaCl 180 g Na_2HPO_4 27 31 g and NaH_2PO_4 $2H_2O$ 4 86 g are dissolved in distilled water and the final volume adjusted to 2 litres This will keep for months without deterioration in a well stoppered bottle In preparing hypotonic solutions for use it is convenient to make first a 1% solution from the 10% stock solu tion by dilution with distilled water Dilutions equivalent to 0 85 0 75 0 65 0 60 0 55 0 50 0 45 0 40 0 35 0 30 0 20 and 0 10% NaCl are convenient test concentrations Intermediate concentrations such as 0 475 and 0 525% NaCl are useful in critical work

The author usually makes up 50 ml of each dilution The solutions keep well at 4 C for some weeks but they should be discarded if moulds develop

Method Heparinized venous blood or defibrinated blood may be used oxalated or citrated blood should be avoided 0 05 ml volumes of the blood to be tested are added to and immediately well mixed in 5 ml volumes of a suitable range of hypotonic solutions The tubes are allowed to stand at room temperature (20) for at least 30 minutes then remixed and

0 45% NaCl	54–96% hæmolysis
0 50%	3?–88%
0 55%	5–70%
0 60%	0–40%
0 65%	0–19%
0 70%	0–9%
0 75%	0 2 %
0 85%	0–0%

AUTOHÆMOLYSIS (Incubation at 37 C for 24 and 48 hours)

Method Sterile defibrinated blood is used Four 2 ml samples are delivered into sterile 5 ml screw capped bottles They are placed in the incubator and left undisturbed for 24 hours The contents of each bottle are then gently mixed by inversion Two of the bottles are then replaced at 37 C and incubated for another 24 hours The contents of the remaining two bottles are pooled a sample removed for the estimation of osmotic fragility (see above) a further sample used for the estimation of the P C V and the remainder centrifuged to obtain the supernatant serum

The amount of spontaneous lysis is estimated by means of a photoelectric colorimeter As a rule it is convenient to make 1 in 25 or 1 in 50 dilutions of the incubated serum in N/10 HCl [1] An appropriate dilution of the pre incubation serum is used as a blank and a 1 in 100 or 1 in 200 dilution of the whole blood in N/10 HCl serves as a standard The percentage hæmolysis allowing for the change in packed cell volume resulting from incubation is calculated as follows (Selwyn and Dacie 1954) —

$$\text{Percentage hæmolysis} = R_T \frac{\left(\frac{100 - PCV_T}{100}\right)}{R_o \times 4} \times 100 = R_T \frac{100 - PCV_T}{R \times 4}$$

R = reading in colorimeter of diluted whole blood
R_T = serum at time T
(i e at 24 or 48 hours)
PCV_T = packed cell volume at time T

Normal Range of Autohæmolysis

Lysis at 24 hours 0 to 0 5%
Lysis at 48 hours 0 4 to 3 5%

[1] Hydrochloric acid rather than ammoniacal water is used as diluent as sometimes a certain amount of methæmoglobin is formed

tary hypertonic solutions to each hypotonic solution at the end of 45 minutes in order to arrest the hæmolysis but this refinement seems unnecessary in practice

Further details of the factors which affect and control the hæmolysis of erythrocytes in hypotonic solutions are given by *Ponder (1918)* Guest (1948) and by Hendry (1948 1949)

Normal Range of Osmotic Fragility (at 20 C and pH 7 4)

0 30% NaCl	97–100% hæmolysis
0 35%	90–99%
0 40%	50–90%
0 45%	5–45%
0 50%	0–5%
0 55%	0

Median corpuscular fragility (M C F) = 0 40–0 445% NaCl

Osmotic Fragility after Incubation at 37 C for 24 Hours

Method Defibrinated blood should be used care being taken to ensure that the sample is sterile Duplicate 2 ml volumes of blood are incubated in sterile 5 ml screw capped bottles (It is useful to set up the samples in duplicate so that in the rare event of a sample being infected as shown by the hæmoglobin being markedly reduced the whole experiment need not be spoilt) After 24 hours the contents of the two bottles normally are pooled after thoroughly mixing the sedimented corpuscles in the overlying serum and the fragility estimated as previously described As the fragility will be found to be markedly increased it is advisable to set up additional hypotonic solutions containing 0 70 and 0 80% NaCl as well as a tube containing 0 90% NaCl In addition a solution equivalent to 1 2% NaCl should be used for sometimes as in hereditary spherocytosis lysis may take place in *0 9% NaCl in this case the supernatant of the tube containing* 1 2% NaCl can be used as the blank in the colorimetric estimation

The incubation fragility test is conveniently combined with the estimation of the amount of spontaneous autohæmolysis (see later) The normal range of osmotic fragility after 24 hours at 37 C is as follows —

0 20% NaCl	91–100% hæmolysis
0 30%	80–100%
0 35%	72–100%
0 40%	65–100%

above are suspected it is well to deliver some blood from the syringe directly into a large volume of saline warmed to 37° C and to wash the corpuscles without delay

When samples are sent by post it is best to send separately (a) serum (separated at 37 C) and (b) whole blood to which sufficient acid citrate dextrose (A C D) solution has been added to prevent coagulation

Storage of Samples Serum is best stored at − 20 C or below in small (1 to 2 ml) volumes Erythrocytes can be kept for as long as 2 to 3 weeks at 4 C if A C D is used as anticoagulant or for longer periods if frozen at − 20 C in citrate glycerol mixtures (Chaplin Crawford Cutbush and Mollison 1954) They cannot be kept for more than a few hours when washed and suspended in saline

Preparation of Erythrocyte Suspensions Erythrocytes should be washed in three changes of a large volume of 0 85% saline before use If cold agglutinins are present it may be necessary to wash the cells in saline warmed to 37° C in order to obtain a smooth suspension After the final washing the test suspension is made by adding to saline in a graduated centrifuge tube an appropriate volume of packed corpuscles using a straight pipette fitted with a rubber teat and filling this to the mark from the bottom of the deposit of centrifuged corpuscles The suspensions should not be made until they are required for use

Suspensions in Albumin These can be simply made by preparing a 1% suspension of the corpuscles in saline and measuring from this the approximate volume that will be needed into a small centrifuge tube After centrifugation the supernatant is removed as completely as possible with a Pasteur pipette This is replaced with the same volume of 20% bovine albumin (Armour)

Reading Agglutination and or Hæmolysis

Agglutination may be read macroscopically as in antiglobulin tests carried out on tiles microscopically as in antibody titrations in albumin or macroscopically using a concave mirror as in reading the results of cold agglutinin titrations In each case the results are scored as follows —

++++ is the strongest reaction e g almost complete agglutination in tile tests occurring in a matter of seconds and resulting in tube tests in a button of cells which remains undispersed when the tube is inverted ± is a weak reaction unquestionably different from the control + ++ and +++ are intermediate reactions of increasing strength

A difference between the rates of hæmolysis of normal and abnormal blood can also be readily appreciated as a rule if blood allowed to clot undisturbed is incubated at 37 C for 24 and 48 hours Normally only small amounts of lysis are visible at the end of 48 hours incubation It is impossible however with this method to record the results quantitatively

MECHANICAL FRAGILITY

Either defibrinated or heparinized blood may be used The first step is to adjust the P C V to 45% by withdrawal or addition of serum or plasma as may be necessary 2 ml volumes are then delivered into two 80 × 10 mm tubes of about 5 ml capacity Four glass beads about 4 mm in diameter are added to the blood and the tubes sealed with tightly fitting rubber bungs They are then rotated at 33 r p m for 60 minutes at room temperature At the end of this time the contents of the two tubes are pooled and 1 in 100 dilutions of the blood are made in N/150 ammonia and normal saline respectively A 1 in 100 dilution in saline of a pre rotation sample is used as a blank and the dilution in N/150 ammonia corresponds with 100% lysis The amount of lysis is then determined in a photoelectric colorimeter using a green (Ilford 625) filter

It is useful to set up duplicate samples of a normal blood as a control whenever the mechanical fragility of a pathological blood is being estimated

The significance of the mechanical fragility test is considered on p 28

Normal range = 2 to 5% hæmolysis

SEROLOGICAL METHODS USEFUL IN THE INVESTIGATION OF THE ACQUIRED HÆMOLYTIC ANÆMIAS AND HÆMOLYTIC DISEASE OF THE NEWBORN

Collection of Samples of Blood and Serum The minimum essential requirements are patient s serum separated from blood allowed to clot at 37 C and a suspension of freshly withdrawn erythrocytes If high titre cold antibodies are suspected it is advisable to deliver the patient s blood directly into a container (e g a 1 oz screw capped glass bottle) previously warmed to 37 C If this is done unhæmolysed serum can be regularly obtained The patient s erythrocytes may be obtained from oxalated citrated or heparinized blood or from blood allowed to clot at 37 C If cold antibodies active at room temperature or

two hours and then centrifuged and washed in three changes of saline warmed to 37° C

Preparation of Cold antibody absorbed Normal Sera Normal human serum from which the normal incomplete cold antibody has been absorbed is a necessary reagent in the titration of incomplete cold antibodies It is prepared by adding the serum to an equal volume of washed packed group O or group A_2 corpuscles and allowing the mixture to stand at 0 C in crushed ice for one hour The absorption must be repeated until normal group O erythrocytes sensitized at a 5% concentration in the serum no longer give positive reactions with antiglobulin sera Two to three absorptions are usually sufficient

DETECTION OF INCOMPLETE ANTIBODIES

The Antiglobulin (Coombs) Test

Direct Qualitative Test The patient s erythrocytes are washed three times in a large volume of saline A 10 to 15% suspension of corpuscles in saline is then made One drop of this is mixed on a translucent tile with a drop of antiglobulin serum diluted to the point of maximum activity (see below) A further drop of the patient s cell suspension added to a drop of saline acts as a control The suspensions are gently rocked from time to time and are viewed with the naked eye or with a hand lens At the end of five minutes or at the most seven minutes the results are read illuminating the tile from below by means of an electric lamp Normal unsensitized corpuscles and corpuscles previously weakly sensitized in an anti D serum should be suspended in the antiglobulin serum alongside the test corpuscles and serve as controls the former suspension acts as a control for the specificity and the latter as a control for the sensitivity of the reaction

Direct Quantitative Test The qualitative test described above is deficient in two respects it gives only a rough idea of the strength of sensitization and it assumes that corpuscles sensitized with different types of antibody react equally readily with a single dilution of antiglobulin serum which is not the case (see p 238) The author therefore routinely uses a simple quantitative antiglobulin test in the investigation of cases of suspected hæmolytic anæmia

Method Serial fourfold dilutions of the antiglobulin serum are made in saline by means of a drop method One drop of each dilution (usually 1 in 4 to 1 in 4 096) is delivered by means of a fine Pasteur pipette serially on to a large opalescent tile one

In microscopic preparations ± agglutination is recorded when uniformly distributed but widely separated small agglutinates (3 to 6 cells) are present in a sea of unagglutinated corpuscles a ± reaction in a test tube read macroscopically with a concave mirror is a distinct granularity persisting after inverting the tube compared with the control saline suspension In antiglobulin reactions ± agglutination may not be obvious until five minutes have elapsed

The *agglutinin* titre is recorded as the reciprocal of the highest final serum dilution (after allowing for the addition of the corpuscles) in which there is ± agglutination

Hæmolysis is read qualitatively after centrifuging the suspensions and comparing the colour of the supernatant with that of the control ++++ represents complete hæmolysis ± is definite but weak hæmolysis compared with the control + is a pale red supernatant and ++ and +++ deep red supernatants

The *hæmolytic titre* is given by the reciprocal of the highest final serum dilution causing ± hæmolysis

Hæmolysis can be read off quantitatively if sufficient supernatant is available by diluting volumes of the supernatants in N/150 ammonia (so as to give a sufficient volume to fill the colorimeter cell) and making comparisons with a 100% standard in a photoelectric colorimeter using a green (Ilford 625) filter Serum or serum dilutions to which no corpuscles have been added serve as blanks

Preparation of Antibody sensitized Erythrocytes It is sometimes useful to prepare as standard control reagents erythrocytes sensitized with a warm antibody and a cold antibody respectively

Warm antibody sensitized cells can be made by suspending one volume of a 50% suspension of washed D positive corpuscles in 9 volumes of an incomplete anti D serum and allowing the suspension to stand at 37 C for two hours before centrifuging and washing It has to be determined by experiment to what extent (if any) the anti D serum should be diluted to give (a) maximally sensitized cells (as determined in optimally diluted antiglobulin serum) and (b) weakly sensitized cells just agglutinable by antiglobulin serum at the end of five minutes exposure

Cold antibody sensitized cells Group O erythrocytes are suspended in a normal serum in the proportion of one volume of a 50% suspension of washed corpuscles to 9 volumes of fresh normal serum (known to contain an adequate amount of incomplete cold antibody) The suspension is chilled at 0 C in crushed ice for

and leave behind antibody components agglutinating erythrocytes sensitized with other types of antibody They found however that the absorbed sera did not retain their specificity very well—sera for instance that had been completely absorbed were found to redevelop to some extent after storage even at — 20 C the ability to react with the cells used for absorption The method is however useful in investigating antiglobulin reactions when the type of antibody is not known

Method It is essential to wash thoroughly the sensitized erythrocytes used for absorption Crawford and Mollison (1951) recommended testing the washings with sulphosalicylic acid until the test (for protein) became negative and then to give one further washing The washed cells should then be tested with the anti globulin serum to make sure that they still react strongly

One volume of antiglobulin serum is then added to one volume of packed washed erythrocytes and the mixture left at room temperature for one hour The mixture is then centrifuged and the supernatant carefully removed with a Pasteur pipette and added to a further volume of packed washed cells (It is con venient to wash the cells used for absorption in several tubes so that when the supernatant saline is removed after the final washing the packed cells at the bottom of the tubes can be used without any further manipulation)

The absorptions should be repeated until the antiglobulin serum diluted to its point of maximum activity no longer agglutinates the cells used to absorb it With anti Rh sensitized cells as many as eight absorptions may be needed (using two volumes of cells to one volume of antiglobulin serum (Crawford and Mollison 1951)) but with cold antibody sensitized cells fewer absorptions will usually prove sufficient

In practice it is not always necessary to absorb the antiglobulin serum completely in order to determine whether two antibodies are reacting with the same component in the serum For instance a reaction apparently of the cold antibody type (see p 238) may be compared with that of known cold antibody sensitized cells by twice absorbing 1 in 4 dilutions of the antiglobulin serum with each type of sensitized cell and then testing each absorbed serum with both types of cell using a sample of the unabsorbed serum as a control If the antibodies are reacting with the same compo nent the intensity of the agglutination of both types of cell by the antiglobulin serum will be diminished irrespective of the type of cell used for absorbing the serum

drop of saline serves as a control One drop of a 10 to 15% erythrocyte suspension is added to each dilution of the anti globulin serum and to the saline control The suspensions are then mixed in succession using the corner of a glass slide starting with the control and finishing with the highest concentration of the antiglobulin serum The results are read after five to seven minutes and scored from ++++ to ± according to the scheme outlined on p 481 Illustrative reactions are given in Table 11 (p 238)

The author finds the tile technique convenient for the results are clear cut and easily read and agglutination develops quickly The antiglobulin reaction of course can be carried out in tubes This is the best method when only small volumes of cells are available For example when an agglutinin titration has been carried out using a 1% suspension of corpuscles the cells can be washed in the original tubes after the agglutination has been read and an equal volume of appropriately diluted antiglobulin serum added to the cell deposit After resuspension and incubation for *30 to 60 minutes at 37 C agglutination can be assessed micro* scopically or macroscopically using a concave mirror

The γ globulin Neutralization Test

As referred to on p 235 it is possible to distinguish two main types of antiglobulin reactions (1) a *reaction inhibited by* adding very small concentrations of γ globulin to the antiglobulin serum (the γ globulin type) and (2) reactions inhibited only by adding much greater quantities of γ globulin (the cold antibody type)

The test is carried out as follows Fourfold dilutions of a 4% solution of human γ globulin are made in saline ranging in concentration from 1 in 4 to 1 in 4 096 Equal volumes of a potent antiglobulin serum diluted 1 in 4 in saline are added to each dilution of the γ globulin After a pause of not less than five minutes the neutralized or partially neutralized samples of anti globulin serum are used to agglutinate the test corpuscles on an opalescent tile as described above Erythrocytes sensitized by anti D and by the incomplete cold antibody present in normal serum respectively should be used as control suspensions (see Table 10 p 236)

Antiglobulin Reactions using Absorbed Antiglobulin Sera

Crawford and Mollison (1951) showed that it was possible to absorb antiglobulin sera with one type of sensitized erythrocyte

The Specificity of the Antibodies It is now realized that not all the warm auto antibodies of acquired hæmolytic anæmia are non specific—some have a specificity within the Rh system (see p 233) In dealing with warm antibodies it is wise therefore to use if possible corpuscles of known Rh genotypes e g CDe/CDe cDE/cDE and cde/cde cells and *not* Rh positive or Rh negative cells chosen at random according to whether the patient is Rh positive or negative However in screening tests for the presence or absence of antibodies group O CDe/cDE corpuscles may be used Tests for antibodies in the serum should not be considered negative unless the serum has been tested with a panel of corpuscles covering all the known blood group antigens

The Concentration of Erythrocytes and the Duration of Sensitization If supplies of serum permit it is probably best to use at least five drops for each test and add to the serum one drop of a 20 to 30% suspension of washed normal erythrocytes Incubation at 37 C or the selected temperature should be prolonged for at least two hours

The Temperature of the Saline and Number of Times the Cells should be Washed after Sensitization Erythrocytes sensitized by warm antibodies can be washed with saline warmed to 37 C or with saline at the temperature of the laboratory Three washings in a large volume of saline are necessary to remove non antibody protein which would neutralize the antiglobulin serum Excess washing has the theoretical objection of possibly removing antibody from the cells Erythrocytes sensitized by cold antibodies *must* be washed in saline warmed to 37 C in order to elute agglutinins

The Optimum Dilution of Antiglobulin Serum As shown on p 238 erythrocytes sensitized by warm antibodies are generally although not invariably agglutinated most strongly in relatively highly diluted antiglobulin sera e g at dilutions between 1 in 16 and 1 in 256 erythrocytes sensitized by cold antibodies on the other hand are most strongly agglutinated in concentrated potent antiglobulin sera e g at a dilution of 1 in 2 or 1 in 4 When dealing with an unknown antibody it is recommended therefore that at least two dilutions of antiglobulin serum be used e g 1 in 4 and 1 in 64

Recommended Procedure for the Detection and Characterization by the Antiglobulin Reaction of Antibodies in the Sera of Patients with Acquired Hæmolytic Anæmia

Based on the considerations outlined in the preceding paragraphs the following suspensions should be set up —

Indirect Antiglobulin Tests

The following points have to be considered when attempting to detect by means of the antiglobulin reaction antibodies in the serum of patients suffering from acquired hæmolytic anæmia the optimum temperature for sensitization the optimum *p*H whether it is necessary for fresh serum to be present the specificity of the antibody or antibodies and the appropriate type of normal erythrocytes that must be used the concentration of erythrocytes in the serum and the duration of sensitization the temperature at which the erythrocytes should be washed and the optimum dilution of the antiglobulin serum

The Optimum Temperature If it is not yet known whether the patient's antibody is a cold or a warm one it is best to set up duplicate suspensions at 37° C. and at room temperature (20° C.) (A test can *not* be considered to have been carried out strictly at 37° C. unless the cell suspension and serum are warmed to this temperature before mixing and the cell serum suspension is diluted in a large volume of warm saline as a preparation for centrifugation before removal from the water bath.) Tests set up at 0° C. to 2° C. give as a rule information of less value as positive results are produced by the incomplete cold antibodies present in normal sera

Optimum pH Little is gained as a rule by acidifying sera containing warm antibodies The sensitizing ability of pathological incomplete cold antibodies however is often markedly enhanced by acidification Ten per cent by volume of N/5 or N/4 HCl should be added therefore to a sample of the patient's serum before the cell suspension is added (see Table 21 p 263)

The Necessity for Fresh Serum Cold antibodies fail to sensitize erythrocytes to antiglobulin serum if the serum used for the sensitization has been previously heated at 56° C. for 5 minutes or longer Actually there is reason to believe that all four fractions of complement are required for the antibody to become irreversibly fixed to the corpuscles (see p. 261) In carrying out tests for incomplete cold antibodies in sera which may be deficient in complement it is necessary therefore to add to a sample of the serum one or more volumes of normal serum (from which the low titre normal incomplete cold antibody has been absorbed) (see Table 19)

The absorption and fixation of warm antibodies is *not* influenced by the presence of complement and tests therefore can be carried out even on heat inactivated sera

The sera should next be absorbed with the three types of cells Usually it is well to retain a sample after one absorption as well as after three or four absorptions The absorptions are carried out by adding the serum to an equal volume of packed washed cells and centrifuging the mixture after one hour at 37° C The absorbed sera are then tested with each type of cell (see Dacie and Cutbush 1954) If the results seem to indicate that the antibody is say anti c the serum must be tested with a panel of c positive and c negative cells chosen to contain a wide range of other antigens so that the specificity of the antibody may be confirmed The serum should also be absorbed with as many different samples of cDE/cDE CDe/CDe and cde/cde corpuscles as practicable Other blood group antigens can be tested for in the same general way

Tests should be carried out using the agglutination of trypsinized corpuscles in addition to the antiglobulin reaction as an indicator of antibody activity

Titrating Antibodies by the Indirect Antiglobulin Method

(a) *Warm Antibodies* Doubling or four fold dilutions of the patient s serum are made in saline so as to give serum dilutions ranging from undiluted serum to 1 in 1 024 To each tube is added an equal volume of a 2% suspension of washed group O CDe/cDE corpuscles After two hours at 37° C the tubes are inspected for agglutination (if any) and the cells washed three times in a large volume of saline

If supplies of the serum are sufficient the author prefers to use 75 × 100 mm tubes and relatively large (i e 0 25 ml) volumes for the titration Sufficient washed cells will then be available for the agglutination in antiglobulin serum to be carried out on a tile in the ordinary way If small volumes have to be used it is then best to add the antiglobulin serum to the deposits of washed corpuscles in the original tubes and to read the agglutination microscopically or with a concave mirror after 30 to 60 minutes at 37 C

(b) *Cold Antibodies* Cold antibodies can be titrated by the antiglobulin method only if normal serum is used as a diluent instead of saline (see Table 19) The normal serum should first be absorbed at 0 C so as to remove the normal incomplete cold antibodies it probably contains (see p 483) The method otherwise is the same as for the titration of warm antibodies except that the test should be carried out at 20 C as well as at 37 C and at

Tube (1) patient s serum 5 vol (5 drops) + 20 to 30% suspension of normal group O CDe/cDE erythrocytes 1 vol (1 drop) Incubate for 2 hours at 37 C
(2) as Tube (1) but at 20 C
(3) as Tube (1) but with the serum previously acidified with a one tenth volume of N/4 HCl
(4) as Tube (3) but at 20 C
(5) as Tube (1) but with an equal volume of fresh normal serum added to the patient s serum
(6) as Tube (5) but at 20 C
(7) as Tube (5) but with the sera acidified with a one tenth volume of N/4 HCl
(8) as Tube (7) but at 20 C
(9) as Tube (3) but using patient s serum which has been inactivated at 56 C for 30 minutes
(10) as Tube (9) but at 20 C
(11) as Tube (1) but with normal serum instead of the patient s serum
(12) as Tube (11) but at 20 C
(13) as Tube (3) but with normal serum instead of the patient s serum
(14) as Tube (13) but at 20 C

All the tubes are allowed to stand at 37 C (or 20 C) for at least two hours the cells being gently resuspended in the serum from time to time At the end of two hours the tubes are inspected for agglutination and hæmolysis The cells are washed in three changes of saline and agglutination tests then carried out on an opalescent tile as described on p 483 using a potent antiglobulin serum diluted 1 in 4 and 1 in 64

If antibody is detected the next step is to titrate it and determine if possible its specificity (see below)

The Determination of the Specificity of an Antibody

Only an outline of the necessary procedures can be attempted here It is essential to have available a panel of normal erythrocytes the blood groups and types of which have been determined as completely as possible Group O cDE/cDE corpuscles are particularly valuable

The first step in determining the specificity of an antibody (if it is a warm one) is to test its ability to react with group O CDe/CDe cDE/cDE and cde/cde cells respectively and to note any differences in the intensity of sensitization or agglutination

fold dilutions of the patient s serum are made in saline so as to give serum dilutions ranging from undiluted serum to serum diluted 1 in 1 024 An equal volume of a 1% suspension of trypsinized corpuscles is added to each tube and to a control tube containing saline only Agglutination is read macroscopically using a concave mirror or microscopically after two hours in the water bath at 37 C (For a description of the use of trypsinized corpuscles in the titration of cold antibodies see below)

DETECTION AND TITRATION OF IN SALINE AGGLUTINATING (COMPLETE) ANTIBODIES

Warm Antibodies *Complete* warm antibodies are rarely detected but not unknown in the sera of patients with acquired hæmolytic anæmia They are titrated by making doubling or four fold dilutions of the patient s serum in saline and adding to each tube an equal volume of a 1% suspension of washed group O CDe/cDE erythrocytes Agglutination is read off macroscopically with the aid of a concave mirror or microscopically after incubation for two hours at 37 C

Cold Antibodies (*a*) *Using Normal Corpuscles* Doubling or four fold dilutions are made in saline so as to give serum dilutions ranging from 1 in 2 to 1 in 2 000 (or higher) Equal volumes of a 1% solution of normal group O corpuscles are added to each tube

The suspensions are allowed to stand for two hours at 20 C (room temperature) and the agglutination then read off macroscopically with the aid of a concave mirror after gently inverting the tube two or three times to resuspend the sedimented corpuscles The suspensions are then remixed and the rack of tubes placed in the refrigerator at 2 to 4 C After chilling for at least two hours the agglutinin titres are re read as quickly as possible before the tubes have had time to warm up appreciably Finally the rack of tubes is placed in the water bath at 37 C and the cells re suspended They are examined for agglutination after a further one hour s incubation

(*b*) *Using Trypsinized Corpuscles* Trypsinized corpuscles are agglutinated by cold antibodies more quickly and more intensely than are normal corpuscles The agglutinin titre is increased perhaps four fold and the upper thermal limit for agglutination raised Titrations are carried out in exactly the same way as with normal corpuscles

lower temperatures or temperatures between 20 C and 37 C if desired After sensitization and reading the agglutinin and hæmolysin titres *the cells are washed in three changes of saline* warmed to 37 C The antiglobulin serum should be used at a dilution of 1 in 4 The titration should be carried out at *p*H 6 5 to 7 0 with acidified normal serum as diluent as well as at *p*H 8 0 using unacidified normal serum

Detection and Titration of Incomplete Warm Antibodies using Albumin and Serum albumin Media

Direct Tests Highly *sensitized corpuscles undergo* auto agglutination when suspended in undiluted normal serum or in 20% albumin The intensity of agglutination is probably a measure of the degree of sensitization of the corpuscles

Indirect Tests Antibodies may be detected by adding to one volume of the patient s serum one volume of a 1 to 2% suspension of normal group O CDe/cDE corpuscles in 20% albumin The presence or absence of agglutination is read microscopically after the suspension has been incubated at 37 C for two hours It is essential to set up a suspension of the test cells in a normal serum as a control for doubtful agglutination

Warm antibodies may be titrated by making doubling or four fold dilutions of the patient s serum in normal serum and adding to each tube an equal volume of the normal corpuscles suspended in 20% albumin The tubes are incubated for two hours at 37 C and the results read microscopically

Detection and Titration of Incomplete Antibodies using Trypsinized Erythrocytes

Preparation of Trypsin Solution Crystalline trypsin (Armour) is very satisfactory A 1% solution is made by weighing out a few mg of the powder and adding the appropriate volume of *N/20 HCl—the solution keeps for a week or more at 4 C* A 0 1% solution is then made by diluting 1 part of the stock solution with 9 parts of isotonic *p*H 7 7 phosphate buffer (1 63% Na_2HPO_4 anhyd 90 5 parts 2 34% NaH_2PO_4 $2H_2O$ 9 5 parts) 0 2 ml of packed washed normal group-O CDe/cDE corpuscles is then added to 1 ml of the 0 1% trypsin solution The mixture is incubated for one hour at 37 C and the trypsinized corpuscles are then washed in at least two changes of saline

Antibody Titration (Warm Antibodies) Doubling or four

In the case of P N H corpuscles however it is important *not* to acidify the serum

A temperature of 20 C is about the optimum for this type of hæmolysis (see p 2?7) and there seems no advantage in incubating at 37 C after sensitizing at 20 C It is probably unwise to chill the cell suspensions at 2 C and then warm to 37 C in some cases less lysis is obtained in this way than by simply allowing the suspensions to stand at 20 C

DETECTION AND TITRATION OF THE DONATH LANDSTEINER HÆMOLYSIN

Qualitative Donath Landsteiner Test Samples of the patient s blood are delivered directly into two tubes or bijou bottles previously warmed in the 37 C water bath One sample is left to clot at 37 C the other is placed immediately in crushed ice at 0 C and left undisturbed for 30 minutes The tube or bottle is then replaced in the water bath at 37 C without disturbing the clot The samples are finally re examined when the clots have retracted In paroxysmal cold hæmoglobinuria the serum expressed by the clot which was chilled before it was warmed should be tinged deeply red with hæmoglobin the serum of the sample kept at 37 C should be entirely free from hæmoglobin

Indirect Donath Landsteiner Test Serum from the patient is obtained from blood allowed to clot undisturbed at 37 C One volume of a 50% suspension of washed normal group O corpuscles is added to 9 volumes of patient s unacidified serum The suspension is chilled in crushed ice at 0 C for 30 minutes then placed in the water bath at 37 C The tube is centrifuged after 60 minutes at 37 C

Hæmolysis visible to the naked eye indicates a positive test In some cases hæmolysis occurs within a minute or so of warming An additional tube containing patient s serum diluted with an equal volume of normal serum should be set up and subjected to the same procedure—this allows for the possibility that the patient s serum is deficient in complement A further control tube may be set up and kept strictly at 37 C throughout this should show no hæmolysis

Titration of the Donath Landsteiner Antibody Doubling or four fold dilutions of the patient s serum are made in fresh normal serum An equal volume of a 2 % suspension of washed group O corpuscles is added to each tube and the tubes then immersed in

DETECTION AND TITRATION OF HÆMOLYTIC ANTIBODIES

Warm Hæmolysins As mentioned on p 242 hæmolytic factors capable of bringing about the hæmolysis of trypsinized or P N H corpuscles are occasionally detected in the sera of patients with acquired hæmolytic anæmia

The hæmolysins may be titrated by making doubling or four fold dilutions of the patient s serum in fresh unacidified normal human serum and adding equal volumes of a 2% suspension of group O trypsinized normal erythrocytes or group O P N H erythrocytes The tubes are incubated for two hours at 37° C and hæmolysis is read visually after centrifuging It is essential that the sera and the cell suspensions be warmed to 37 C before the cells are added to the serum

Cold Hæmolysins (*excluding* the Donath Landsteiner antibody) Sera containing cold antibodies at high titres are potentially hæmolytic (see p 250) Normal corpuscles are as a rule only hæmolysed in acidified sera

Hæmolysis can be reliably demonstrated by adding to 10 volumes of the patient s serum previously acidified with 10% by volume of N/4 HCl 1 volume of a 50% suspension of normal group O erythrocytes A second tube containing patient s serum diluted with three parts of fresh normal serum and then acidified should be set up to allow for the possibility that the patient s serum is deficient in complement Further tubes containing patient s unacidified serum and normal acidified serum should be set up as controls The tubes are left at 20 C for two hours and then gently centrifuged Typically hæmolysis is seen only in the tubes containing patient s acidified serum

The final cell concentration should not be greater than 5% and care should be taken to deliver the cell suspension directly into the serum If the cell suspension comes into contact with the side of the tube this may by itself lead to hæmolysis

Titration of Cold Hæmolysins Hæmolytic high titre cold antibodies can be titrated by making doubling or four fold dilutions of the patient s serum in acidified fresh normal serum (containing 10% by volume of N/4 HCl) and adding to the serum dilutions equal volumes of a 2% suspension of normal group O corpuscles The tubes are allowed to stand for two hours at 20 C They are then centrifuged and inspected for hæmolysis

Trypsinized corpuscles and P N H corpuscles can also be used

−20° C overnight, rewashed and then suspended in two volumes of saline and the *p*H adjusted to 6.6 to 6.8 with N/10 NaOH. The suspended stroma is heated at 60° C for 10 minutes and then rapidly centrifuged for a short while in previously warmed centrifuge cups. The supernatant is finally re-centrifuged at high speed so as to remove all particulate matter.

PREPARATION OF ANTIGLOBULIN SERUM

Several methods may be used, and as only a minority of rabbits produce potent sera, it is advisable to immunize several animals at the same time. Full details are given by Mourant (1952).

The primary stimulation may be carried out by the alum precipitated serum method of Proom (1943) or by Slavin's (1950) method using calcium alginate.

Proom's Method. 25 ml. of human group O serum are diluted with 80 ml. of distilled water and 90 ml. of 10% potash alum added. The *p*H is adjusted carefully to 6.5 with 5N-NaOH using bromthymol blue as external indicator. The preparation is centrifuged and the precipitated protein washed twice with saline containing 1 in 10,000 merthiolate. The final precipitate is made up to 100 ml. with merthiolate saline. 5 ml. of this material are injected intramuscularly into each thigh of the rabbit to be immunized and the injections repeated 14 days later.

Slavin's Method. 1 ml. of human group O serum is mixed with 4 ml. of 4% sodium alginate solution (Calgitex). This is best done in a sterile mortar. The mixture is injected intraperitoneally into a rabbit using a comparatively wide bore needle. Immediately afterwards 2.5 ml. of a 1% aqueous calcium chloride solution are injected by means of a second syringe into the same area.

Hyperimmunization. Irrespective of the method of primary immunization, it is advisable to hyperimmunize the rabbits by injecting whole human serum intravenously, preceded by an intraperitoneal injection (Wootton, 1950). At least four weeks after the primary inoculation 0.5 ml. of human group O serum is injected intraperitoneally. The following day 0.1 or 0.2 ml. of serum, diluted in 1 or 2 ml. of saline, is slowly injected intravenously. The rabbit should be bled five to seven days later. The intraperitoneal and intravenous inoculations may be repeated at intervals of four to six weeks.

Rabbits can usually be bled satisfactorily from their ear veins. The blood is placed at 37° C so that the clot may retract. The

crushed ice at 0° C. After 30 minutes the tubes are placed in the water bath at 37° C and the deposited cells resuspended. They are centrifuged after one hour's incubation. The degree of hæmolysis is recorded as described previously (p. 482).

ELUTION OF ANTIBODIES FROM SENSITIZED ERYTHROCYTES

The preparation of potent antibody-*containing eluates from* the erythrocytes of patients with acquired hæmolytic anæmia is an essential step in the investigation of the specificity of the antibodies (see p. 233). Eluates are probably best made from the erythrocyte stromata. Kidd's (1949) acid elution and Selwyn's (1952) heat elution techniques both yield potent eluates. Further work is required before it can be assumed that all types of antibody *can be equally successfully eluted by* a single technique.

Kidd's (1949) Method. It is convenient to work with the erythrocytes obtained from 20 ml. or more of the patient's defibrinated blood. The erythrocytes are washed at least three times in saline and an equal volume of distilled water is then added to the packed washed cells. The partially lysed erythrocytes are then frozen and thawed at least three times. Finally, a volume of distilled water five times that of the original volume of packed cells is added and the lysed blood then acidified by the dropwise addition of N-HCl until the maximum amount of stroma appears to have been precipitated (*at about pH 5.6 to 5.8*). The precipitated stroma is washed at least six times with M/15 phosphate buffer at *p*H 5.6 to 5.8. Two to three volumes of *p*H 3.2 M/10 citrate-HCl buffer are then added to one volume of packed washed stroma, and the reaction readjusted to *p*H 3.2 to 3.4 by the cautious addition of further N-HCl, using bromphenol blue as an external indicator.

Acid elution is continued for 10 to 15 minutes at room temperature, gently agitating the suspension of stroma from time to time. The suspension is then centrifuged and the supernatant kept. The *p*H is adjusted to 7.4 by the careful dropwise addition of 5N-NaOH using phenol red as an external indicator. *During* neutralization protein is precipitated. This is removed by centrifugation and the supernatant, usually a slightly brown clear solution, should contain the eluted antibodies.

Selwyn's (1952) Method. Stroma is obtained as indicated above and washed until almost hæmoglobin free. It is frozen at

50% hæmolysis This is calculated as follows (Mayer, Eaton and Heidelberger 1946) —

x = the reciprocal of the final serum concentration

$$y = \frac{(\text{observed \% hæmolysis})}{100 - \text{observed \% hæmolysis}}$$

y is plotted for each value of x against x on double log paper

The titre causing 50% lysis is given by the point at which a straight line fitted to the experimental observations cuts the y axis at $y = 1$

Normal Range of Serum Complement (titre) = 70 to 150 units

SCHEME FOR THE SEROLOGICAL INVESTIGATION OF A PATIENT SUSPECTED OF SUFFERING FROM ACQUIRED HÆMOLYTIC ANÆMIA OF THE AUTO ANTIBODY TYPE

It is hoped that the following brief scheme may be of use It has been set out in the form of answers to questions The recommended procedures are outlined in italics

(1) Are the patient's erythrocytes sensitized by auto antibodies?
Direct antiglobulin test

(1a) If the antiglobulin test is positive is the reaction of the warm or cold type?
Quantitative antiglobulin test γ globulin neutralization test

(1b) What is the specificity of the antibodies?
Prepare eluates test by antiglobulin method and with T N cells Determine the exact group and genotype of the patient's erythrocytes as far as is possible

(2) Is there "free" antibody in the patient's serum?
(a) *Cold agglutinin titration at 2° C using N cells*
(b) *Agglutinin titration at 37° C using T N cells*
(c) *Indirect antiglobulin reaction sensitizing at 37° C*
(d) *Qualitative D–L test*

(3) If a high titre cold antibody is present what is its thermal range?
(a) *Cold agglutinin titration at 20° C 30° C and 37° C using N cells*
(b) *Indirect antiglobulin reaction sensitizing at 20° C 30° C and 37° C ± acidification ± heat inactivation of the patient's serum*

separated serum is then freed from suspended cells by centrifugation and inactivated by heating at 56 C for 30 minutes

The serum is then absorbed with human erythrocytes which have been washed with at least six washings in a large volume of saline Two absorptions should be carried out first with an equal volume of packed group O cells and then with group A_1B cells or with a mixture of A_1 cells and B cells The cell serum suspensions should be left for at least one hour at 4 C before centrifuging

The absorbed rabbit serum must be tested for its specificity and potency When diluted 1 in 4 it should not agglutinate washed normal human erythrocytes and it should be capable of agglutinating erythrocytes deliberately weakly sensitized with antibodies such as anti D and the incomplete cold antibody present in normal sera It should be titrated with both types of antibody and optimum dilutions determined for each If the serum is shown to be both sensitive and properly absorbed it may be brought into use It should be stored frozen at − 20 C in volumes not exceeding 2 ml

TITRATION OF SERUM COMPLEMENT

Doubling dilutions of freshly obtained sera are made in saline so as to give serum dilutions ranging from 1 in 8 to 1 in 128 0 5 ml volumes are convenient To each tube of the serum dilutions and to two further tubes containing 0 5 ml of saline and distilled water respectively is then added an equal volume of a 2½% suspension of washed sheep cells sensitized with 1 5 M H D of rabbit anti sheep cell amboceptor (5% washed sheep cells to which has been added an equal volume of saline containing 3 M H D of amboceptor and the mixture allowed to stand at 37 C for 30 minutes) The serum cell suspensions are placed in the 37 C water bath for 30 minutes and then centrifuged

0 5 ml volumes of the supernatants from each tube and from the two control tubes are then added to tubes containing 4 5 ml of N/150 ammonia The amount of hæmolysis in each tube is read in a photoelectric colorimeter using a green (Ilford 625) filter The control tube which contained only saline gives a reading for 0% hæmolysis and the tube containing distilled water a value for 100% hæmolysis

The *complement titre* is recorded as the reciprocal of the serum concentration after the addition of the sheep cells which causes

Method Fresh 0 5 ml samples of normal serum and/or patient s serum are acidified by the addition of one tenth volumes (0 05 ml) of N/5 HCl After careful mixing the acidified serum is placed in the water bath at 37 C and 0 05 ml (or one large drop) of a 50% suspension of patient s washed corpuscles is added to each tube The tubes are centrifuged after incubation at 37 C for one hour In paroxysmal nocturnal hæmoglobinuria the corpuscles in the acidified serum will have undergone definite although incomplete lysis No lysis or at the most a trace of lysis should be visible in the unacidified sample As essential controls additional tubes of unacidified and acidified serum respectively should be set up and normal corpuscles instead of patient s corpuscles added to them No lysis should be visible in either tube

Markedly spherocytic erythrocytes undergo lysis in acidified serum and this must be borne in mind in assessing the significance of a positive result (Dacie 1949) The two types of reaction can be readily differentiated by repeating the test using acidified serum previously inactivated at 56 C for 10 to 30 minutes P N H erythrocytes do not undergo lysis in heated serum the lysis of the spherocytes however is unaffected As is shown in Fig 95 (p 432) it is possible to construct a pH lysis curve if different concentrations of acid are used The optimum pH for hæmolysis is between pH 6 5 to pH 7 0 (pH measurements made after the addition of the erythrocytes to the serum)

The acid serum test is positive (subject to the reservations on spherocytosis mentioned in the previous paragraph) only in paroxysmal nocturnal hæmoglobinuria

THE ESTIMATION OF THE SURVIVAL OF TRANSFUSED ERYTHROCYTES BY THE DIFFERENTIAL AGGLUTINATION METHOD OF ASHBY

Principle of the Method The general principle of the method is to transfuse to a recipient erythrocytes of a different but compatible blood group e g group O corpuscles may be transfused to a group A or B or AB recipient group ON corpuscles to a group OM or OMN recipient and Rh negative corpuscles to an Rh positive recipient The resulting mixture of corpuscles may be separated *in vitro* if they are suspended in a potent agglutinating serum in which the recipient s corpuscles are agglutinated but not those of the donor For example when group O blood has been transfused to a group A recipient the group A corpuscles are agglutinated by an anti A serum but the donor s group O

(3a) Is the antibody hæmolytic ?
  (a) *Titrate with N cells at 20° C using normal serum as diluent ± acidification*
  (b) *Titrate with P N H cells (if available) at 20 C using normal serum as diluent*
  (c) *If D-L antibody present carry out the quantitative D-L procedure sensitizing at 0 C*

(4) If a warm antibody is present what is its specificity ?
  Test with panel of cells of known genotype and carry out absorption experiments

  (4a) Are hæmolytic components present ?
  Titrate at 37 C with N T and P N H cells using normal serum as diluent

(5) Are there any other serological abnormalities ?
  Estimation of serum complement
  Estimation of serum proteins separation by paper electrophoresis Titration of heterophile (anti sheep) antibodies Agglutination and complement fixation tests for anti viral antibodies and antibodies against Streptococcus M G

N cells = normal group O erythrocytes T N cells = trypsinized normal group O erythrocytes P N H cells = paroxysmal nocturnal hæmoglobinuria erythrocytes D-L = Donath Landsteiner

THE ACID SERUM TEST

The acid serum test is used in the diagnosis of paroxysmal nocturnal hæmoglobinuria It is necessary to have samples of the patient s erythrocytes as well as serum from the patient or compatible fresh normal serum The patient s corpuscles can be obtained from defibrinated heparinized oxalated or citrated blood The test can be satisfactorily carried out even on corpuscles which have been stored for several weeks in acid citrate dextrose solution at 4 C

The patient s serum is best obtained by defibrination for if obtained from blood allowed to clot in the ordinary way at 37 C or room temperature it will almost certainly be found to be markedly hæmolysed Normal serum can be obtained either by defibrination or from blood allowed to clot spontaneously at room temperature or at 37 C It is advisable to use a sample of normal serum which is known to be potent in causing the hæmolysis of P.N H corpuscles (see Fig 94 p 423)

Method Fresh 0 5 ml samples of normal serum and/or patient s serum are acidified by the addition of one tenth volumes (0 05 ml) of N/5 HCl After careful mixing the acidified serum is placed in the water bath at 37 C and 0 05 ml (or one large drop) of a 50% suspension of patient s washed corpuscles is added to each tube The tubes are centrifuged after incubation at 37 C for one hour In paroxysmal nocturnal hæmoglobinuria the corpuscles in the acidified serum will have undergone definite although incom plete lysis No lysis or at the most a trace of lysis should be visible in the unacidified sample As essential controls additional tubes of unacidified and acidified serum respectively should be set up and normal corpuscles instead of patient s corpuscles added to them No lysis should be visible in either tube

Markedly spherocytic erythrocytes undergo lysis in acidified serum and this must be borne in mind in assessing the significance of a positive result (Dacie 1949) The two types of reaction can be readily differentiated by repeating the test using acidified serum previously inactivated at 56° C for 10 to 30 minutes P N H erythrocytes do not undergo lysis in heated serum the lysis of the spherocytes however is unaffected As is shown in Fig 95 (p 432) it is possible to construct a pH lysis curve if different concentrations of acid are used The optimum pH for hæmolysis is between pH 6 5 to pH 7 0 (pH measurements made after the addition of the erythrocytes to the serum)

The acid serum test is positive (subject to the reservations on spherocytosis mentioned in the previous paragraph) only in paroxysmal nocturnal hæmoglobinuria

THE ESTIMATION OF THE SURVIVAL OF TRANS FUSED ERYTHROCYTES BY THE DIFFERENTIAL AGGLUTINATION METHOD OF ASHBY

Principle of the Method The general principle of the method is to transfuse to a recipient erythrocytes of a different but compatible blood group e g group O corpuscles may be trans fused to a group A or B or AB recipient group ON corpuscles to a group OM or OMN recipient and Rh negative corpuscles to an Rh positive recipient The resulting mixture of corpuscles may be separated *in vitro* if they are suspended in a potent agglutinating serum in which the recipient s corpuscles are agglutinated but not those of the donor For example when group O blood has been transfused to a group A recipient the group A corpuscles are agglutinated by an anti A serum but the donor s group O

corpuscles remain in a dispersed suspension Similarly when group ON blood is transfused to a group OMN recipient the recipient's corpuscles can be agglutinated by an anti M serum leaving the donor's group ON corpuscles unagglutinated

It is possible to carry out the differential agglutination in a quantitative way and to count the unagglutinated corpuscles with an accuracy hardly less than that for straightforward erythrocyte counts However it is absolutely essential to use a highly avid agglutinating serum This usually restricts the method to tracing the survival of group O blood given to a group A or B or AB recipient using an anti A or anti B serum and of group ON blood given to a group OMN or OM recipient using powdered anti M serum (Lederle) Full details of the use of the differential agglutination method are given by Mollison (1951) The technique now to be described is slightly modified from that described by Dacie and Mollison (1943) Survival curves obtained by the method are illustrated in Figs 21 22 23 and 85

Technique 0 1 ml of venous blood is suspended in 4 9 ml of 3% sodium citrate solution to make a 1 in 50 dilution One volume of the suspension is then added to one volume of the appropriate agglutinating serum in an 80 × 10 mm tube provided with a well fitting rubber bung 0 25 ml volumes are suitable

It is good practice to do the test in duplicate if sufficient serum is available The serum should be used undiluted or diluted in several volumes of saline if necessary to the concentration at which agglutination is maximal as shown by preliminary experiments with the recipient's blood Agglutination of the recipient's cells should be intense before transfusion and there should be very *few free cells if a good serum is used* the best results will be obtained if there are less than 10 000 free cells per c mm when blood containing 5 000 000 erythrocytes per c mm is agglutinated by the technique to be described

The dilution of blood in citrate and serum (now 1 in 100) is left at room temperature for at least two hours and then centrifuged at about 1 500 r p m for one minute The tubes are then quite vigorously shaken so that not only are the unagglutinated cells suspended but the button of agglutinated cells becomes broken up into small but still visible fragments After waiting for not *more* than one minute during which time the largest clumps of agglutinated cells sink to the bottom of the tube the upper three quarters of the suspension consisting of free cells and small clumps only is removed by Pasteur pipette into a fresh tube This tube is corked and the contents centrifuged for one minute as

before The button of deposited cells is then well mixed with the supernatant fluid by a standard procedure—fifty inversions through an angle of 90 to 120 at the rate of one per second A counting chamber is then filled from the upper layers of the cell suspension thus minimizing the number of agglutinates withdrawn

After waiting for at least two minutes for the cells to settle an erythrocyte count is performed in the usual way counting however free cells only The cells (usually tightly agglutinated) in the few clumps which may be seen are ignored The number of unagglutinable (donor) cells present may be expressed in absolute numbers or as a percentage of the number present at the conclusion of the transfusion

If anti M powder is used instead of a liquid agglutinating serum the powder itself is added by means of a small wooden spatula (toothpick) to a 1 in 50 or 1 in 100 dilution of the patient s whole blood in 3% sodium citrate The amount to be added must be determined by trial and error the aim being to achieve almost complete agglutination of M positive cells (i e less than 10 000 free cells per cmm)

DEMONSTRATION OF HEINZ BODIES

Unstained Preparations Heinz bodies may be seen as refractile objects in dry unstained films if the illumination is cut down by lowering the microscope condenser They are also easily seen by dark ground or phase contrast illumination The size of the particles varies from 1 to 2 μ to half the size of the corpuscles One or more may be present in a single cell They are usually close to the cell membrane and in wet preparations they move around within the cells in a slow Brownian movement

Stained Preparations Methyl violet stains the bodies excellently

Equal volumes of blood and 0 5% methyl violet in normal saline are mixed together and after about ten minutes at room temperature films may be made or the suspension of corpuscles viewed between slide and coverslip The Heinz bodies stain an intense purple They also stain with other basic dyes With brilliant cresyl blue they stain less intensely than with methyl violet However they may be readily seen as pale blue bodies in a well stained reticulocyte preparation

If permanent preparations are required the vitally stained films should be fixed by exposure to formalin vapour for 5 to 10 minutes If films are fixed in methyl alcohol the bodies are decolorized

Formalin fixed films may be counterstained with 0 1% eosin or 0 1% safranin after thoroughly washing in distilled water

DEMONSTRATION OF SICKLING

The sickling phenomenon may be simply demonstrated by sealing a thin film of the patient s blood between slide and cover slip by means of a Vaseline and paraffin wax mixture Sickling develops in the sickle cell trait and in the various types of sickle cell anæmia as the oxygen in the preparation is gradually used up In sickle cell anæmia well marked sickling is usually visible after incubation for an hour or less at 37 C in the trait the process is slower and up to 12 hours incubation may be necessary The change can be hastened by the addition of reducing agents to the blood The method recommended by Itano and Pauling (1949) is a reliable one

Method Using a Reducing Agent

Required *A* 0 114M aqueous sodium dithionite ($Na_2S_2O_4$)
B 0 114M aqueous disodium hydrogen phosphate (Na_2HPO_4)

The two reagents are mixed together to give a final *p*H of about 6 8 about 2 volumes of *A* to 3 volumes of *B* are required

The sodium dithionite solution should be made up on the day it is required and the appropriate amount of sodium phosphate solution added just before use

About 0 05 ml of the reagent is added to a very small drop of blood (0 01 ml) on a slide and the mixture immediately covered with a coverslip In sickle cell anæmia the early changes of sickling take place almost immediately holly leaf forms first appear and birefringence becomes visible in 15 to 20 minutes

PHYSICO CHEMICAL METHODS USEFUL IN THE INVESTIGATION OF ABNORMAL HÆMOGLOBINS

Brief accounts will be given (a) of a method for the determination of the rate of denaturation by alkali and (b) of a method of paper electrophoresis applicable to the differentiation of the human hæmoglobins The first stage of either method is the preparation of concentrated stroma free hæmoglobin solutions

Preparing the Hæmoglobin Solutions The patient s erythrocytes are washed twice in 0 85% saline and then once in 1 2% saline To the packed cells is added an equal volume of

distilled water and the mixture is then repeatedly frozen and thawed The resultant viscous solution is treated with a one fifth volume of Cy aluminium hydroxide gel or with washed asbestos pulp prepared from a shredded Ford Sterimat The absorbent and the erythrocyte stroma are then removed by centrifuging in a high speed angle centrifuge (c 18 000 r p m) for one hour at 5 C

The resultant hæmoglobin solution has a concentration of 10 to 15 g per 100 ml and stores well at 5° C in completely filled bottles

Method for the Quantitative Measurement of the Rate of Alkali Denaturation

Principle The alkaline hæmatin is estimated by measuring the increasing optical density of a solution of hæmoglobin in red (640 mμ) light after the addition of alkali As the absorption due to alkaline hæmatin at 600 to 650 mμ is much greater than that of oxyhæmoglobin the development of the alkaline hæmatin can be accurately recorded

Method (White 1954) 4 8 ml of the hæmoglobin solution are placed in a 1 cm cell belonging to a photoelectric colorimeter fitted with a spectral red filter The absorption of the red light is minimal and should give a reading of less than 1 on the colorimeter scale 0 2 ml of N-NaOH is then added to the hæmoglobin solution and rapidly stirred into it Readings on the colorimeter scale are taken at 10 second (or longer) intervals until the reaction is complete

In an adult the denaturation is usually complete in less than 100 seconds in a newborn infant on the other hand a rapid initial rise in density is followed by a slow increase which may not be complete for two and a half hours For comparative purposes the reactions should be carried out at the same temperature

If the rise in density due to the denaturation is plotted against time on semi log paper a straight line is obtained with the blood of an adult containing no fœtal hæmoglobin and the half reaction time can be readily deduced With blood containing fœtal (alkali resistant) hæmoglobin two component curves are obtained an initial steep slope due to the presence of adult hæmoglobin being followed by a slow component due to the fœtal hæmoglobin By extrapolation backwards the percentage of fœtal hæmoglobin can be calculated (Fig 57 p 128) (White and Beaven 1954)

Filter-paper Electrophoresis of Human Hæmoglobins

Principle. Small differences in the iso-electric points of the various human hæmoglobins allow their separation by electrophoresis. The difference in behaviour between adult and fœtal hæmoglobins is slight, but sickle-cell hæmoglobin and hæmoglobin D move more slowly toward the anode than normal hæmoglobin on paper electrophoresis at the alkali side of the iso-electric points. Hæmoglobin C has even less anodic mobility than hæmoglobins S and D under these conditions.

Details of the following technique of separation have been kindly provided by Dr. G. H. Beaven and Dr. J. C. White. The curves illustrated in Fig. 61 (p. 153) were obtained by its use.

Method

Hæmoglobin Solution. Although oxyhæmoglobin, methæmoglobin and carboxyhæmoglobin behave similarly on paper electrophoresis, the carboxy compound is preferable as it is very stable and readily resolved. Solutions of carboxyhæmoglobin are prepared from concentrated stroma-free hæmoglobin (see p. 502) by diluting the hæmoglobin with buffer (see below) to a concentration of 3 to 5 g. per 100 ml. and then saturating with carbon monoxide or scrubbed coal gas. Saturation is best effected by passing the gas through a small flask on the sides of which the hæmoglobin solution is spread out as a film.

Apparatus. Paper strips (Whatman No. 1 or 3 MM) 9 cm. in width and 24 cm. in length are supported on a rectangular perspex frame. The ends of the paper pass down vertically for 8 cm. to dip into the inner troughs of buffer. The inner troughs are connected by glass-wool wicks with buffer in 500 ml. outer troughs into which pass platinum wire electrodes. The troughs are built into the perspex box enclosing the frame and paper, and an air-tight lid is provided.

Buffer. Barbitone buffer at pH 8.6 of ionic strength $\Gamma/2 = 0.05$.

Barbitone sodium	5 g.
Hydrated sodium acetate	3.33 g.
N/10 hydrochloric acid	34.2 ml.
Hydrated copper sulphate	1 mg.
Water to	1 litre

Potential and Current. A potential of 100 to 240 volts is applied across the paper, the current being 5 to 10 milliamps. Dry cells or a stabilized mains D.C. supply are suitable.

Setting up the Test A line is drawn in pencil across each paper strip between the centre of the paper and the cathode end of the horizontal section On each paper the identification of two specimens is marked in the papers are soaked in the buffer blotted and applied across the frame with the paper ends dipping into the inner buffer troughs The lid is placed on the apparatus and equilibration of buffer over the paper allowed to take place for 1 hour A pair of hæmoglobin samples is then applied to the pencilled line on each paper by means of a camel hair brush dipped into the HbCO solutions in two linear marks One specimen on the paper is a standard marker of normal hæmo globin or a known sickle trait or sickle cell hæmoglobin—hæmo globin C mixture etc the other is the unknown solution under test Inclusion of such markers greatly facilitates precise identi fication of unknown specimens as conditions for observation of absolute mobilities are difficult to achieve in paper electrophoresis

The potential is then applied for 16 to 20 hours It is useful though not essential to pass carbon monoxide into the apparatus at the commencement The apparatus is shielded from light and variations in temperature At 20 C separation is good but if the room temperature is much higher it may be necessary to set up the apparatus at a controlled lower temperature

Examination of Results At the end of the separation the strips are removed and hung up to dry in air The separation of the hæmoglobin components is evident to the naked eye the identifica tion of the components being aided by comparison with the markers A rough quantitative comparison of the constituents in mixtures is also possible by eye

For more precise measurement of comparative mobilities and graphical representation of separation into two or more compo nents scanning with some form of densitometer is necessary It is best to fix the hæmoglobins by heating at 110 C and then to stain them by a suitable dye such as naphthalene black The papers are differentiated in acetic acid methanol until the marks are clear and the background very light They are then dried and rendered translucent in a mixture of liquid paraffin and 1 bromonaphthalene

A direct recording of the density along the prepared paper strip can be made by an automatically recording densitometer (Laurence 1954) (see Fig 61 p 153)

The relative proportions of the components into which a mixture has been resolved can be determined by measuring the area under each peak in the density tracing by means of a planimeter

SPECTROSCOPIC EXAMINATION OF BLOOD FOR METHÆMOGLOBIN AND SULPHÆMOGLOBIN

Method Blood is diluted 1 in 5 or 1 in 10 with water and then centrifuged The clear solution is examined in a glass cell or tube It is important that the greatest possible depth or concentration of solution (consistent with visibility) should be examined and that a careful search should be made (with varying depths or concentrations of solution) for absorption bands in the red part of the spectrum (620–630 mμ) If bands are seen the solution should be treated with a drop of yellow ammonium sulphide A band due to methæmoglobin will then disappear if sulphæmoglobin is present its band persists For comparison laked blood may be treated with potassium ferricyanide solution which will cause the formation of methæmoglobin A sample of sulphæmoglobin may be prepared from blood (10 ml of 1 in a 100 dilution) by adding to it phenylhydrazine hydrochloride solution (0 1 ml of a 0 1% solution) and a drop of water saturated with hydrogen sulphide The unknown and the known pigments may then be compared in a reversion spectroscope

The absorption band in the red due to methæmoglobin is at the wavelength 630 mμ and that due to sulphæmoglobin at 618 mμ (cf methæmalbumin at 624 mμ)

SCHUMM S TEST

The serum (or plasma) is covered with a layer of ether A one tenth volume of saturated yellow ammonium sulphide is then added and mixed with the serum which is viewed with a spectroscope If methæmalbumin is present an ammonium hæmochromogen will be produced which has an intense narrow absorption band in the green (at 558 mμ)

ESTIMATION OF PLASMA HÆMOGLOBIN

The method described below is a modification of that of Bing and Baker (1931)

Principle Benzidine in acid solution and hydrogen peroxide together in the presence of hæm pigments give a green colour which changes to blue and finally to reddish violet The intensity of the colour may be compared in a photoelectric colorimeter with that produced by solutions of known hæmoglobin content Methæmalbumin and hæmoglobin are measured together

Every effort must be made to prevent hæmolysis during the collection and manipulation of the blood. A clean venepuncture is essential; a relatively wide bore needle should be used and the syringe, first rinsed with sterile saline, should fill spontaneously with blood. When the required amount of blood has been withdrawn, the needle should be detached and nine parts of blood added to one part of 3.8% sodium citrate. All glassware must be scrupulously clean.

Method. 0.1 ml. of plasma (or a larger volume of an appropriate dilution of the plasma, see later) is added to 2 ml. of the benzidine reagent and 1 ml. of the hydrogen peroxide solution in a large test tube. A control tube in which 0.1 ml. of distilled water is substituted for the plasma, and a standard tube containing a known amount of hæmoglobin are also set up.

The mixtures are allowed to stand at room temperature for one hour and then 20 ml. of a 20% by volume aqueous solution of glacial acetic acid are added to each tube. The colours developed are compared in a photoelectric colorimeter using the colour developed by the control tube as a blank. A blue green (Ilford 624) filter is suitable.

If the hæmoglobin content of the plasma to be tested is abnormally high, the plasma should be diluted until it is just visibly tinged with hæmoglobin.

Normal Range. 1–4 mg. hæmoglobin per 100 ml. plasma (Crosby and Dameshek, 1951).

Reagents. *Benzidine Solution.* 0.5 g. of pure benzidine dihydrochloride (Merck) is dissolved in 15 ml. of hot (not boiling) distilled water, and then 25 ml. of 95% ethyl alcohol and 10 ml. of glacial acetic acid added. The benzidine solution will keep for several weeks in a dark bottle at 2° to 5° C.

Hydrogen Peroxide. 0.6% solution prepared by diluting a 3% (10 vols.) solution with distilled water before use.

DEMONSTRATION OF HÆMOSIDERIN IN URINE

The urine is centrifuged and the supernatant removed and replaced by an equal volume of a freshly made solution of 1% potassium ferrocyanide in 1% HCl (made by mixing equal volumes of 2% potassium ferrocyanide and 2% HCl). The deposit is resuspended in the acid potassium ferrocyanide solution and allowed to stand at room temperature for 5 to 10 minutes. The suspension is then re-centrifuged. The deposit is transferred to a slide, covered with a coverslip and examined under the micro-

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must be multiplied by the dilution factor Obviously icteric plasma should be diluted in the first instance

Calculation

Photoelectric Colorimeter

$$\text{Bilirubin (mg per 100 ml)} \begin{cases} = \dfrac{\text{Reading of test}}{\text{Reading of standard}} \times 0.04 \times \dfrac{100}{1} \\ = \dfrac{\text{Reading of test}}{\text{Reading of standard}} \times 4 \end{cases}$$

Solutions

Stock Standard Methyl red Solution 290 mg of pure methyl red are dissolved in 100 ml of glacial acetic acid

Methyl red Standard (2 9 mg per litre at pH 4 63) 1 ml of the standard is placed in a litre flask together with 5 ml of glacial acetic acid Water is added and 14 4 g of crystallized sodium acetate are washed into the flask When solution is complete the volume is made up to 1 litre with water

Diazo Reagent This is made by mixing two solutions *A* and *B*

Solution A is made by dissolving 1 g of sulphanilic acid in 250 ml of N hydrochloric acid and making the volume up to 1 litre with water

Solution B contains 0 5 g of sodium nitrite in 100 ml water

The diazo reagent is made freshly before use by mixing 0 3 ml of solution *B* with 10 ml of solution *A*

Alcohol 85% ethyl alcohol

Buffer 3 6 g of disodium phosphate (Na_2HPO_4 $12H_2O$) in 100 ml water

Ammonium Sulphate Saturated solution

ESTIMATION OF UROBILINOGEN IN FÆCES

Principle of the Method The stercobilin pigments of the fæces are reduced to urobilinogen which is extracted with water and the solution treated with Ehrlich s dimethylaminobenzaldehyde reagent to produce a pink colour which can be compared with either a natural or an artificial standard

Method (King 1951)

Approximately 1 5 g of well mixed fæces are transferred by means of a glass rod to a 6 × 1 in test tube (This is easily done by weighing the glass rod before and after) 9 ml of water are

scope using the 4 mm objective Hæmosiderin if present appears in the form of isolated or grouped blue staining granules usually from 1 to 3μ in size

If a permanent preparation is required the unstained urinary deposit is allowed to dry in the air It is then stained by the same technique as is used to stain blood films for siderocytes The deposit is first fixed by dipping the slide in methyl alcohol for 10 to 20 minutes It is stained in freshly prepared acid potassium ferrocyanide solution for 10 minutes in the 56 C water bath The slide is then washed in running water for 20 minutes rinsed in distilled water and finally counterstained with 0 1% safranin or eosin

ESTIMATION OF SERUM BILIRUBIN

Principle of the Method The serum (or plasma) is treated first with diazotized sulphanilic acid and then with ammonium sulphate and alcohol to precipitate protein The red colour produced is compared in a photoelectric colorimeter with that of an artificial standard (methyl red—2 9 mg per litre at pH 4 63) The colour of this solution accurately matches the colour obtained when 0 04 mg of bilirubin is treated with the diazo reagent in a final volume of 10 ml

Method (King 1951)

Test 1 ml of plasma or serum is treated in a centrifuge tube (or better in a glass stoppered tube) with 0 5 ml of diazo reagent If the diazo reagent is carefully layered above the plasma and the tube allowed to stand for a few moments a positive direct van den Bergh reaction (if present) may be seen at the liquid junction 0 5 ml of saturated ammonium sulphate and 8 ml of 85% ethyl alcohol are added The mixture is stoppered thoroughly mixed allowed to lie on its side for 30 minutes and then filtered Under these conditions the dilution of the plasma closely approximates 1 in 10

The colour of the clear filtrate is compared with the standard mentioned above (= 0 04 mg of bilirubin in a volume of 10 ml) The comparison is made with a green filter (Ilford 624)

If the concentration of azo bilirubin in the test appears to be more than twice that in the standard a suitable dilution of the original plasma with a phosphate buffer solution (see below) should be made and the procedure repeated Since this involves a dilution of the plasma (e g 1 in 3 or 1 in 10) the resultant reading

must be multiplied by the dilution factor Obviously icteric plasma should be diluted in the first instance

Calculation

Photoelectric Colorimeter

$$\text{Bilirubin (mg per 100 ml)} \begin{cases} = \dfrac{\text{Reading of test}}{\text{Reading of standard}} \times 0\ 04 \times \dfrac{100}{1} \\ = \dfrac{\text{Reading of test}}{\text{Reading of standard}} \times 4 \end{cases}$$

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Method (King 1951)

Approximately 1 5 g of well mixed fæces are transferred by means of a glass rod to a 6 × 1 in test tube (This is easily done by weighing the glass rod before and after) 9 ml of water are

then added and the glass rod used to stir the mixture until it is well emulsified 10 ml of ferrous sulphate solution are added and well mixed and then 10 ml of 2 5N sodium hydroxide The mixture is allowed to stand for 2 hours with occasional stirring and is then filtered

Test 2 ml *of filtrate* (≡ *0 1 g of fæces*) are placed in a 100 ml measuring cylinder and 2 ml of Ehrlich s reagent are added After mixing and allowing to stand for 10 minutes 6 ml of sodium acetate solution are added (plus an equal or greater volume of water if the colour of the test is much greater than that of the standard)

Standard (≡ 0 00387 mg urobilinogen per ml) 1 ml of phenolphthalein standard in a 100 ml volumetric flask is treated with 5 ml of sodium carbonate solution diluted to the mark with water and mixed

Blank 2 ml *of the fæces filtrate* 2 ml *of 6N hydrochloric acid* and 6 ml of sodium acetate are treated in the same way as the test

The test and standard samples are compared in a photoelectric colorimeter using a yellow green (Ilford 625) filter

The blank reading is subtracted from that of the test

Calculation

Urobilinogen mg (per 100 g fæces)

$$\begin{cases} = \dfrac{\text{Reading of test} - \text{Reading of blank}}{\text{Reading of standard}} \times 0\ 00387 \times V^* \times \dfrac{100}{0\ 1} \\ = \dfrac{\text{Reading of test} - \text{Reading of blank}}{\text{Reading of standard}} \times V^* \times 3\ 87 \end{cases}$$

* Final volume of the coloured solution (ml)

Solutions

Ferrous Sulphate 20 g $FeSO_4$ $7H_2O$ dissolved in water and made up to 100 ml

2 5N sodium hydroxide (approx) 10 g of NaOH dissolved in water and made up to 100 ml

Ehrlich s Dimethylaminobenzaldehyde Reagent 0 7 g *p* dimethylaminobenzaldehyde dissolved in a mixture of 150 ml concentrated hydrochloric acid and 100 ml water

Sodium Acetate Saturated solution of sodium acetate

6N hydrochloric Acid 60 ml of concentrated acid diluted to 100 ml *give approximately 6N HCl*

Standard Phenolphthalein Solution 50 mg phenolphthalein

dissolved in 100 ml of alcohol and diluted 1 in 100 in alkaline solution as described above This phenolphthalein standard has a colour similar to that given by 0 387 mg urobilinogen in 100 ml when treated by the above procedure or to 0 00387 mg in 1 ml

Sodium Carbonate 15 g Na_2CO_3 dissolved in water and made up to 100 ml

The Standard of Watson Schwartz Sborov and Bertie (1944) An alternative standard solution which more nearly matches the urobilinogen test consists of 5 mg of Pontacyl Carmine 2B and 95 mg of Pontacyl Violet 6R150 per cent dissolved in 1 litre of 0 5% acetic acid When 10 ml of this solution are diluted with 60 ml of 0 5% acetic acid a colour is obtained which is equivalent to that of 0 6 mg of urobilinogen in 100 ml when treated with Ehrlich s reagent

TESTS FOR UROBILINOGEN AND UROBILIN IN URINE

The amounts of urobilin which are usually present are too small to impart a colour to the urine of normal persons Most of the pigment is present as the colourless urobilinogen which readily becomes urobilin on oxidation Urines containing large amounts of urobilin are reddish in colour

A Qualitative Test (King 1951)

Zinc Test To 5 ml of urine are added 2 drops of N/10 iodine solution followed by 5 ml of a 10 % suspension of zinc acetate in alcohol The mixture is allowed to settle and in the clear supernatant a green fluorescence becomes apparent if urobilin or urobilinogen is present If a spectroscope is available the fluid may be examined for the broad absorption band (due to urobilin) at the green blue junction

Quantitative estimations of urobilinogen may be carried out using Ehrlich s reagent dimethylaminobenzaldehyde as described for the estimation of urobilinogen in faeces

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